Breeding for Disease Resistance in Farm Animals

3rd Edition

Edited by

Stephen C. Bishop
Roger F.E. Axford
Frank W. Nicholas
and John B. Owen

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I Principles and Methods

1 Introduction

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Breeding for improved disease resistance has become perhaps the major challenge facing animal geneticists. The benefits of successfully improving the resistance of animals to an infectious disease are manifold, including improved animal welfare, increased efficiency and productivity, and hence a reduced environmental footprint, reduced reliance on other disease-control measures and improved public perception. However, breeding for disease resistance raises many technical challenges. Further, despite its apparent benefits, its sustainability is often questioned due to the potential of pathogen or parasite evolution; and the role of host genetics within integrated disease-control systems is often unclear. This 3rd edition of *Breeding for Disease Resistance in Farm Animals* addresses many of the pertinent questions relating to the role of host genetics in disease control, with a number of case-specific scenarios explored.

When considering breeding for disease resistance, it is necessary to be clear and consistent in the concepts and terminology being used, to ensure that readers from disparate disciplines have a common level of understanding of the topic. This Introduction covers many of the broad concepts necessary for all readers to appreciate the topic, with a particular focus on recent developments in genomics and their application to disease genetics. We hope it will make the individual chapters more enjoyable.

Infectious Disease: the Context

Infectious diseases in livestock result in high economic losses in both developed and developing countries. They also have potentially major impacts on the safety of animal products (especially for food safety), animal welfare and the public perception of livestock production industries. Further, due to the impacts of climate change and globalization, i.e. increased movement of people and

products, new disease threats continue to emerge (Foresight Project, 2006). For these reasons, the management of infectious disease is of critical importance to livestock sectors worldwide and is the subject of considerable ongoing research.

Disease-control strategies include both prevention and cure, and may include decisions affecting the animal (e.g. vaccination, culling diseased animals, selection of resistant animals), the pathogen (e.g. chemotherapy) or the environment (e.g. biosecurity, sanitation). With the recent development of extensive high-throughput genomic tools that enable dissection of host responses to infection and comprehensive descriptions of host genetic variation, research efforts have increasingly turned to quantifying the genetic control of the host–pathogen interaction, as well as identifying single nucleotide polymorphisms (SNPs) associated with resistance. Much is promised in terms of identifying critical host genes that may lead to novel non-genetic means of combating parasites/pathogens, e.g. new vaccine targets or even entirely novel approaches to disease management derived from a greater understanding of the underlying biology. Much is also promised from the use of SNP genotypes as another source of information to be incorporated into estimated breeding values (EBVs) for use in conventional selection programmes for 'disease resistance' (without knowing anything of the actual genes involved). However, these promises need to be critically evaluated and some of the concepts are discussed below.

The use of host genetic variation, including SNPs associated with resistance, to help control disease should always be considered as part of a larger disease-management strategy. While host genetic manipulation will be a valuable tool for some diseases, for other diseases it may be of low priority in relation to other disease-control strategies, or possibly not even appropriate. Therefore, careful consideration is required to determine when breeding for disease resistance is appropriate, and for which diseases it is possible to obtain the necessary genetic and phenotypic information to achieve this.

Genetic Variation in Disease Resistance

Present-day species of farm livestock have inherited a complex genome from their wild progenitors. Yet, despite the proliferation of phenotypic variation in breeds within species, molecular studies reveal that differences at the DNA level between extant breeds and their wild relatives are rather small. A feature of both modern and progenitor breeds is the ubiquity of host genetic variation in disease resistance. This is largely a function of the co-evolution of the host and its parasitic pathogens (Khibnik and Kondrashov, 1997) – a continual battle to achieve an ecological equilibrium enabling both species to survive.

Co-evolution models help to explain the existence of host genetic variation in resistance, and further insight into the continued existence of such variation can be gained by extending co-evolution models to combine genetic theory with epidemiology. Several factors are important. First, selection pressures, especially those for disease resistance, will differ across time and environments.

Second, in the case of epidemic diseases, natural selection will not make populations completely resistant to infection. As natural selection moves a host population towards resistance, the selection pressure for resistance decreases because a certain proportion of susceptible animals can be carried without exposing the population as a whole to risks of epidemics (Bishop and MacKenzie, 2003). Once the number of genetically susceptible animals falls below this level, selection pressure for resistance ceases. Third, modern domestic livestock populations have been selected for other characteristics, with disease impacts masked by non-genetic control measures.

Evidence for host genetic variation in aspects of disease resistance has been documented for more than 50 diseases, in all major domestic livestock species (Bishop, 2005). Such genetic variation covers all types of parasite and pathogen, and the genetic architecture of host resistance ranges from single major genes to polygenic in the extreme. Almost certainly there is host genetic variation in resistance to almost every disease: those cases not yet documented are merely awaiting discovery.

Care must also be taken in the definition of the term 'disease resistance', as it is often used to mean many different things. Infection may be defined as the colonization of a host by organisms such as viruses, bacteria, protozoa, helminths and ectoparasites, whereas disease describes the pathogenic consequence of infection. Disease resistance is used generically to cover resistance to infection, i.e. a host's ability to moderate the pathogen or parasite lifecycle, and also resistance to the disease consequence of infection. Sometimes the terms tolerance or resilience are used to describe a host's ability to withstand pathogenic effects of infection.

When and How to Breed for Disease Resistance

The large number of diseases faced by animals in every production system raises policy and logistical challenges. It is not easy to select for resistance to more than a few diseases simultaneously; nor is it desirable to do so as it could potentially remove considerable selection pressure from existing selection goals. Additionally, adequate control strategies will often exist for many diseases, making justification for expensive and long-term breeding programmes rather weak.

Approaches to addressing the problem of disease prioritization have recently been proposed. Davies *et al*. (2009) describe an approach in which diseases are ranked in terms of their importance and also in terms of their amenability to genetic selection. This approach immediately highlights key target diseases. A further consideration, which isn't considered by Davies *et al*. (2009), is the benefit of genetically improving the resistance of the population as a whole. This concept may be captured through genetic-epidemiological models (Bishop and Stear, 2003). Briefly, the consequences of genetic change in the resistance of a population of animals to an infectious disease depend upon the transmission pathways of infection. Furthermore, the outcomes of selection should be measured at the population level, rather than the individual

animal level, e.g. addressing questions such as 'is an epidemic likely to occur in this population and, if so, how severe would it be?' The outcomes are very non-linear in relation to host genotype, and depend upon the starting point. For example, a moderate improvement in resistance to viral disease might either solve the disease problem or make no impact at all, depending on the nature of the disease and the initial level of resistance of the host. Such considerations are outlined in Chapter 3, and they provide a means of prioritizing diseases for research and designing implementation strategies.

Careful consideration has to be made before embarking on a breeding programme for enhanced resistance to a specific disease. First, a need to genetically improve resistance has to be established. This will include an appraisal of the importance of the disease and the possible shortcomings or non-sustainability of current control measures. Second, the benefits of achieving improved resistance, including the epidemiological benefits, need to be assessed. As with all traits in a breeding programme, animal breeders will need to be convinced that including disease resistance in the breeding goal adds to the overall value of genetic progress to a greater extent than if disease resistance were not taken into account, i.e. the net benefits of including disease resistance outweigh the opportunity cost of reduced progress in other traits.

In principle, selection for disease resistance can be performed using either traits that indicate the response of animals to an infectious challenge or DNA markers. The latter has the obvious advantage of not requiring exposure to infection in order to rank animals, and for diseases with severe impacts this may in fact be the only viable option. As a consequence, much of the current research in disease genetics is aimed at finding such markers, as described below. Selection based on animal phenotype will be feasible in cases of endemic diseases which pose a predictable challenge to animals. Important examples, discussed in this edition, include mastitis and nematode infections in ruminants.

Application of Genomics to Disease Genetics

Most of the case studies described in this volume describe the application of genomics to the target disease in order to disentangle between-host variation in disease resistance or response to infection. It was of course the discovery of the structure of DNA in 1953 that heralded the beginning of the molecular revolution. This subsequently led to an understanding of the structure of genes, the identification of genetic markers and the development of sequencing technologies which, within the last decade, have led to complete genome sequences for several livestock species as well as the ability to detect polymorphisms throughout the genome.

Some of the major outcomes of the molecular revolution are the ability to: (i) detect variation in base sequence in most regions of most chromosomes of a species, i.e. to discover and define DNA markers; (ii) determine the base sequence of segments of DNA, i.e. to sequence genes and identify likely mutations underlying genetic variation seen between hosts; and (iii) detect which

genes are being transcribed in a particular tissue at a particular time, i.e. to detect and quantify gene expression. This latter process is critical in understanding host responses to infection and in moving towards an understanding of precisely *how* hosts differ in their resistance to a disease of interest. Together, these steps give us the tools to dissect, understand and utilize host genetic variation in disease resistance. We will consider each of these steps in turn.

Identification and utilization of DNA markers

It was the advent of recombinant DNA technologies, particularly the polymerase chain reaction (PCR) technique, that removed constraints on marker availability. This in turn allowed large-scale marker discovery, development of dense linkage maps and ultimately high-throughput genotyping. Microsatellite markers were initially responsible for the expansion in linkage maps of domestic livestock, being co-dominant multi-allelic tandem repeats spread throughout the genome. Microsatellites comprise a simple sequence, usually AC/GT, which is typically repeated between 10 and 50 times and their alleles are defined by the number of simple-sequence repeats. The highly polymorphic nature of microsatellites made them informative for genome mapping studies, for identification or exclusion of parents and for genetic diversity studies. However, there is a current tendency for microsatellites to be replaced by SNPs as the marker of choice for most genomic applications. Although SNPs usually have only two alleles, and hence are less informative at the individual locus than microsatellites, they are more amenable to scaling up for high-throughout genotyping and ultimately they are more cost-effective. Further, they are more numerous than microsatellites, occurring perhaps every kilobase of sequence, enabling much finer mapping of disease-causing loci. Ultimately, SNPs are the basic building block of much of the observed genetic variability and they provide testable candidates for causal mutations.

Panels of microsatellite or SNP markers, and the dense linkage maps derived from these markers, enable identification of regions of chromosomes containing genes that contribute to variation in a trait of interest, such as disease resistance. Such regions are called quantitative trait loci (QTL). With microsatellite and sparse SNP marker panels, QTL are generally identified by linkage studies in defined pedigrees, exploiting linkage between the DNA markers and the unknown causal mutation, and hence their co-segregation within families. However, such studies give poor resolution on the location of the QTL. With the availability of dense SNP panels (see below), association studies based on population-wide linkage disequilibrium (LD) between DNA markers and the causal mutation have become popular. Where the phenotypic and genotypic data allow, a combination of linkage and LD mapping may allow more precise definition of haplotypes containing the causal mutation (e.g. Druet and Georges, 2010).

In terms of breeding animals for disease resistance, individual QTL or marker associations can be exploited by marker-assisted selection (MAS). The utility of markers for this purpose will depend upon the proportion of the genetic variation that they explain; it is likely that there will be only a relatively small

number of diseases where selection on single markers or QTL is warranted as resistance to most diseases appears to be somewhat polygenic. However, examples are given in this book where individual markers or QTL do justify selection, including scrapie resistance in sheep (Chapter 4), resistance to infectious pancreatic necrosis in salmon (Chapter 8) and two forms of *Escherichia coli* resistance in pigs (Chapter 11). In most cases where resistance is polygenic, a far more powerful approach is the identification of a set of SNPs that together account for a large portion of the genetic variation in a trait. Although these may be discovered during a genome-wide association study (GWAS), the key is to identify SNPs that jointly are able to predict the observed genetic variation irrespective of their individual significance (Goddard and Hayes, 2009). Selection based on this approach is generally known as genome-wide selection (GWS).

Genome-wide selection has been enabled by the advent of dense SNP arrays in most farm animal species. The standard array size is now more than 50,000 (i.e. 50 k) SNPs, covering all regions of the genome, although in dairy cattle arrays of up to 800 k SNPs are now available. In putting GWS into practice, the aggregate animal genotype is calculated from the sum of all SNPs whose effect (estimated from the difference between the two homozygotes) exceeds an agreed value. This initial stage, where the prediction equations are developed, requires phenotyping and genotyping of many (thousands of) animals. Subsequently, individual aggregate genotypes or breeding values can be predicted for genotyped animals that do not have phenotypes. This has two obvious major advantages for disease genetics: first, extensive phenotyping and genotyping with all available SNPs is not required every generation (although it is required from time to time, for 'recalibration'); and, second, it enables the capture of genetic information from disease breakdowns in the field.

The density of SNP arrays required for effective GWAS and GWS is a complex function of effective population size, the extent of LD and the genetic architecture of the trait of interest, including its heritability. Given the relatively small effective population sizes of most livestock breeds and long stretches of LD compared with humans, 50 k arrays are generally considered adequate for calibrating GWS. However, in most circumstances 50 k arrays will not capture all the genetic variation, and greater densities will increase accuracy and enable more precise GWAS studies.

Genomic studies will generally require large-scale collection of field data to ensure sufficient power to perform either GWAS or GWS. Of particular interest in a disease context is the fact that the disease resistance phenotype is often recorded as a binary trait, e.g. affected or not, leading to a poor ability to identify genetically resistant animals when the disease prevalence is low. A natural solution to this problem is to utilize case-control designs, making the accuracy of the genomic predictions of resistance or disease risk independent of the disease prevalence (Daetwyler *et al*., 2008). Coupled with this is the concern that field disease data are noisy, i.e. the exposure status of animals is often unknown and the diagnostic test may be poor, with animals often misclassified. However, it has been shown (Bishop and Woolliams, 2010) that incomplete exposure or poor diagnostic test specificity or sensitivity simply reduce selection accuracy in rather

predictable ways, and detectable genetic variation in the face of poor phenotype ascertainment is indicative of perhaps stronger underlying genetic control.

Finally, in addition to providing tools to enable selection for increased disease resistance, fine-mapping of QTL can lead to identification of the actual coding sequence(s), i.e. the mutation, underlying the QTL. This knowledge is of value in itself, as it can be used to investigate the biochemistry and physiology underlying the trait of interest, e.g. disease resistance.

Genome sequencing

We have now reached the stage where whole-genome sequence assemblies are, or will soon be, available for most of the species discussed in this book. These assemblies are by no means perfect, and considerable efforts are being made to improve their accuracy. In addition to giving considerable insight into the structure of genomes and their evolutionary similarities, they provide a very powerful point of reference for genome mapping. In other words, they are the ultimate genetic/genomic map.

From the perspective of understanding and utilizing genetic variation in disease resistance, it is the re-sequencing of the genome that will be critically important. Currently, re-sequencing specific genes or short genome regions in individual animals is an important step in SNP discovery and in the identification of potential causal mutations. Ultimately, however, it is the sequencing of the entire diploid genome of each member of a population of animals that will give the greatest insight. Not only will this provide the scientific community with the ultimate identification of genetic variation, but it will also provide the tools for GWS that will be even more effective than is currently possible with SNP arrays. Although the costs of re-sequencing individuals are likely to be high, costs are rapidly falling and the US$1000 human genome sequence is within reach. From an animal breeding perspective, these extra costs will be offset by the increased accuracy of GWS from complete sequences and, possibly more importantly, the likelihood that the calibrated prediction equations will remain accurate over many more generations than typically observed for SNP arrays (Meuwissen and Goddard, 2010).

Detecting gene expression

Although genome sequences give a complete description of the genetic variation that an individual has, they do not directly describe when and how genetic differences affect observed phenotypes. One of the approaches towards addressing this issue has been the detection of gene expression. This approach has been used to quantify how animals respond to stimuli such as a pathogen challenge, and to compare animals that may differ either genetically or phenotypically in their resistance.

Many gene expression studies have been performed using expression arrays. With this technique the array, or chip, contains the coding sequence of

all known genes in a species or a subset of genes of interest. From animals participating in an experiment, RNA is extracted from a tissue at a particular time, reflecting the genes being expressed in that tissue at the time of sampling. A cDNA copy of the RNA is then made, and the genes that are being expressed in that tissue at the time of sampling are determined by estimating the extent to which the cDNA hybridizes to each gene on the chip. In many laboratories this expression of array approach is now being replaced by high-throughput sequencing approaches, whereby many RNA transcripts (from the same sample) are sequenced, and bioinformatic techniques are used to relate each specific transcript back to known genes. Relative gene expression is then determined from the number of each unique transcript that has been sequenced.

Irrespective of the means of determining gene expression, comparison of gene expression in the same tissue at the same time from animals with contrasting genotypes or phenotypes (e.g. resistant and susceptible) may be used to gain insight into the genetic basis of resistance. Interesting questions include not only differences between animals in how they respond to infection, but also underlying gene expression differences between animals prior to infection. Invariably, individual experiments are somewhat limited in their scope, although conducting meta-analyses on sets of gene-expression data has the potential to identify biological pathways involved in the trait of interest (e.g. disease resistance). In addition to being important for understanding the possible consequences of selection for disease resistance, the increased understanding of the biological basis of traits such as response to infection or disease resistance has the potential to lead to non-genetic interventions to enhance disease control.

Major Opportunities

For a number of reasons, the major opportunities that present themselves for breeding for disease resistance will tend to be endemic diseases. For example, it is endemic diseases that are most likely to meet the criteria of being important diseases for which other disease control strategies are, by definition, failing. Such diseases also enable easy capture of phenotypic data upon which to base selection or calibrate genetic markers.

While many epidemic diseases (e.g. foot and mouth disease, avian influenza) have a higher profile than most endemic diseases, typically they do not lend themselves to breeding for disease resistance. First, animal phenotype collection for such diseases is inherently problematic due to strict containment requirements for such diseases. More critically, selecting animals for enhanced resistance, while conceptually a useful insurance policy, is likely to conflict with current disease-control strategies which are based on eradication. In summary, the genetic approach must complement rather than conflict with other disease-control strategies.

Most of the specific infectious diseases covered in this edition are either endemic in the production systems within which they are important, or potentially endemic. It is interesting to note a close correspondence between the diseases identified by Davies *et al*. (2009) as being the top candidates for disease genetic

studies and the diseases covered in detail in this edition. In fact, the only high-ranking disease that is not covered in detail in this edition is coccidiosis in poultry.

Sustainability

The sustainability of genetic improvements in disease resistance is often questioned, specifically in terms of whether the parasite or pathogen will evolve to overcome genetic changes in the host. A more tractable question is whether genetic selection poses a greater or lesser risk of parasite/pathogen evolution than other forms of disease control, such as chemotherapy or vaccination. These issues are considered in more detail by Gibson and Bishop (2005), and some pertinent points are made here.

Much is to be learnt from indigenous livestock breeds. The disease-resistance genes of indigenous breeds that have evolved under endemic disease challenge will, by definition, be involved with biological mechanisms against which the pathogens have been unable to evolve resistance. Such mechanisms are more likely to be resistant to the future evolution of the pathogen. As such, utilization of genetic resistance of indigenous livestock genetic resources has a higher likelihood of having long-term sustainability and will be the application of choice where feasible.

In general, disease-control strategies that combine different approaches are likely to be more sustainable, as pathogens/parasites with a mutation allowing them to escape one strategy will still be susceptible to other forms of control. Thus, the combined use of host-genetic resistance with other control strategies will often be more sustainable than the use of any one control strategy alone. On the same theme, host-genetic resistance based on several genes will often be more sustainable than resistance based on a single gene. Further, selection pressures on the pathogen/parasite caused by host-genetic resistance will usually be lower than with therapeutic or vaccine interventions. Therefore, host-genetic resistance should be more sustainable than disease-control interventions that place a strong selection pressure on successful pathogen/parasite mutants.

These brief considerations should not detract from the possibility that detrimental pathogen/parasite evolution may occur. This is particularly the case for pathogens such as bacteria or viruses that have a large population size and a short generation interval relative to the host. Also, there is a risk with selection based on genetic markers alone that parasite evolution may go unnoticed; hence marker-based selection may be more risky than phenotype-based selection. In practice, however, the greatest pressure on the pathogen to evolve will only occur after genetic improvement is widely disseminated in the livestock production system, and the pressures on the parasite will generally be less than those created by other modes of disease control.

Future Challenges and Threats

We live in a changing world, with many forces for change impacting on livestock production sectors. Most obvious are the pressures due to climate change

and current pressures resulting from changes in the world economic landscape. Together, these pressures have the potential to change the disease challenges seen in many production systems and reduce the ability of the livestock sectors to respond effectively.

As a broad summary of the climate-change phenomenon, many arid tropical and subtropical regions could become warmer and drier, with substantial water shortages, whereas current temperate regions may become warmer but wetter. Consequences have already been seen in Europe with the arrival of bluetongue as a major disease threat for small ruminants during the summer of 2007.

These threats pose particular challenges for disease geneticists. Because the threats are likely to comprise sporadic epidemic diseases, they represent scenarios that are less tractable for disease-genetic studies as well as being diseases for which the role of host selection is less clear. Nevertheless, much may be learnt from the harvesting of information from sites of disease breakdowns, e.g. using the natural case-control design created by such breakdowns, and interrogating differences between cases and controls using dense SNP arrays. Such information may well contribute to future disease-control strategies.

Gaps in this Edition

The diseases covered in this edition are a subset of the disease challenges faced by farmed livestock. However, they are representative of the main diseases for which breeding for resistance is a realistic possibility. Despite this, some readers may be disappointed to find some diseases missing from this edition.

A highly ranked disease identified by Davies *et al*. (2009) that is not covered here is coccidiosis, an economically important intestinal parasitic disease of poultry caused by *Eimeria* infection. Due to likely future difficulties in controlling *Eimeria* infections in Europe, arising from the withdrawal of coccidial drugs and increasing levels of drug resistance, alternative control measures for this disease will become a priority. Host genetic variation in resistance is well established in inbred lines (Bumstead and Millard, 1992; Smith *et al*., 2002) and outbred populations (Pinard-van der Laan *et al*., 1998; Zhu *et al*., 2003). With the availability of more powerful genomic tools we expect coccidiosis to become a target disease for animal geneticists, and hence become a topic for a chapter in a future edition.

Further diseases that are not covered are fly strike in sheep as well as bovine tuberculosis and paratuberculosis. Fly strike was covered extensively in the previous edition and it remains an important issue in many sheep-producing regions. However, apart from the study of Smith *et al*. (2008), little research on genetic variation in resistance has been published since the previous edition. We are aware of research currently being conducted with genomic tools, and we look forward to reinstating the chapter on fly strike in the next edition. Both tuberculosis and paratuberculosis are important endemic diseases in many cattle production systems, and are currently

the focus of considerable research. At the time of planning this edition, research into host genetic control of these two diseases was generally at an early stage, and inclusion in a book focusing on breeding for resistance could not be justified. However, two major studies quantifying genetic variation between dairy cattle in tuberculosis resistance have recently been published (Bermingham *et al*., 2009; Brotherstone *et al*., 2010), and this disease will surely become a central focus of genomic studies over the next few years.

Aims and Structure of the Book

In addition to covering a wide range of diseases in some detail, this book also aims to give readers necessary basic information and knowledge of the disciplines that underpin breeding for disease resistance. The hope is that readers who approach the topic with only a sketchy knowledge of these underlying disciplines will be armed with sufficient information to enable a full appreciation of chapters of their interest.

This edition has a number of changes from the previous edition, reflecting changes in available technologies and research. As described above, much of the focus of disease-genetic studies is now based on the detection of SNP associations for their own sake, with possibilities for genome-wide selection following as a natural consequence. Further, identification of causal mutations often leads on to powerful functional studies. These concepts underpin much of the science described in this volume. Viral diseases have also been the focus of much research in the last decade. Consequently, the section on viral diseases has been expanded from a single chapter to a series of host-specific chapters. Lastly, the inclusion of a chapter devoted to viral diseases in salmonids reflects the growing importance of aquaculture as a major contributor to rural economies in many countries.

We hope that you enjoy this new edition.

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2 The Immune System

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Summary

This chapter summarizes and updates our current understanding of the immune systems of farm animal species. It highlights the relatively recent understanding that innate immune responses are specific to classes of pathogen and drive downstream adaptive immune responses, critical for immunological memory. Examples are given where disease resistance has been shown to involve immune mechanisms and, in a few cases only to date, genes that encode molecules involved in immune responses. The concept of increased immune robustness to challenge a wide range of pathogens, by selecting for increased innate immune responsiveness, is also discussed. Our ability to understand immune responses in farm animal species and to map genes involved in disease resistance has improved greatly with the availability of genome sequences for these species and the accompanying post-genomic technologies. The current challenge is to deal with the consequent data deluge, but prospects for breeding for disease resistance at the level of the immune response are exciting.

Introduction

The past decade has seen a revolution in our understanding of the immune response to infection and disease, facilitated by the availability of genome sequences not just for biomedical model species such as man and mouse, but also now for farm animal species such as the chicken, cow, horse and pig. The crucial role and specificity of the innate immune response in driving and controlling adaptive immune responses to particular pathogens is now beginning to be understood and manipulated. The roles of the effector cells of the innate immune response (natural killer (NK) cells and neutrophils) and other lymphocyte subsets ($\gamma\delta$ T cells), and interactions between these and antigen-presenting cells, particularly dendritic cells (DCs), are also better characterized. Another major advance is in our understanding of the regulation of adaptive immune responses, particularly in the repertoire of CD4 T cell subsets, which has expanded beyond

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the original Th1/Th2 paradigm (Mosmann *et al*., 1986; Mosmann and Coffman, 1989) to include regulatory subsets (e.g. Treg, Th3, Tr1; reviewed in Cohn, 2008; Zhu and Paul, 2008) and other effector subsets (Th17, Harrington *et al*., 2005; Th9, Dardalhon *et al*., 2008; Veldhoen *et al*., 2008).

Our understanding of these cellular subsets and the responses in which they are involved for farm animal species naturally lags behind that in biomedical model species. However, the availability of farm animal genome sequences has allowed the identification of the repertoires of immune molecules present in these species and facilitates the rapid development of the reagents necessary to begin to understand their functions. Already it is becoming clear that immune responses in mammals fit broadly into the biomedical species blueprint, but that differences do occur in the detail. For non-mammalian species, however, things can be radically different. For example, the chicken has a different repertoire of immune genes, molecules, cells and tissues compared with mammals. However, the basic principle of innate immune responses driving appropriate adaptive immune responses to clear initial infection and provide immunological memory remains constant for all vertebrate species so far studied that have an adaptive immune response.

Selection for improved immune resistance is complex. In general, few single genes or gene products have been shown to influence disease resistance, with some exceptions such as CCR5 and CXCR4, which are associated with resistance to HIV (reviewed in Kuhmann and Hartley, 2008), and the single dominantly expressed chicken MHC class I gene (Wallny *et al*., 2006), which is associated with resistance to a number of poultry viruses. It is now commonly accepted that disease resistance is likely to be a multifactorial trait. Indeed, selection for an improved adaptive immune response against a particular pathogen may compromise the ability to mount an appropriate response against a different pathogen. However, there is potential to select for increased innate immune responses, leading to increased immune robustness, or the ability to resist infection by wide spectra of pathogens.

Innate Immunity

The innate immune response was for many years considered as a non-specific, barrier-immune function, and in one sense as an evolutionary relic of primordial immune systems predating the development of adaptive immune responses. However, it is now apparent that the innate immune response is specific, if not to individual pathogens then to certain classes of pathogens, and that the innate response drives adaptive immune responses appropriate to combat infection with a particular pathogen. The innate immune response has its own receptors (pattern recognition receptors (PRRs)) and effector cells (e.g. neutrophils, NK cells and DCs), produces cytokines and chemokines that drive inflammatory responses and the acute phase response and influence subsequent adaptive responses, and presents pathogen antigen to the adaptive immune response, in the context of the major histocompatibility complex (MHC), via antigen-presenting cells (APCs), in particular the professional APCs, the DCs.

Pattern recognition receptors

Pattern recognition receptors recognize both exogenous and endogenous antigens. The latter are called danger-associated molecular patterns (DAMPs), and include molecules such as uric acid. Of more relevance to the immune response, PRRs also recognize pathogen-associated molecular patterns (PAMPs) (Fig. 2.1). These may be molecules expressed on the surface of pathogens, such as lipopolysaccharide, lipoteichoic acid, flagellin and peptidoglycans, or pathogen nucleic acid, including single-stranded RNA, double-stranded RNA or CpG DNA. PRRs can be broadly divided into two classes, either by function (signalling PRRs and endocytic PRRs) or location (membrane-bound PRRs or cytoplasmic PRRs).

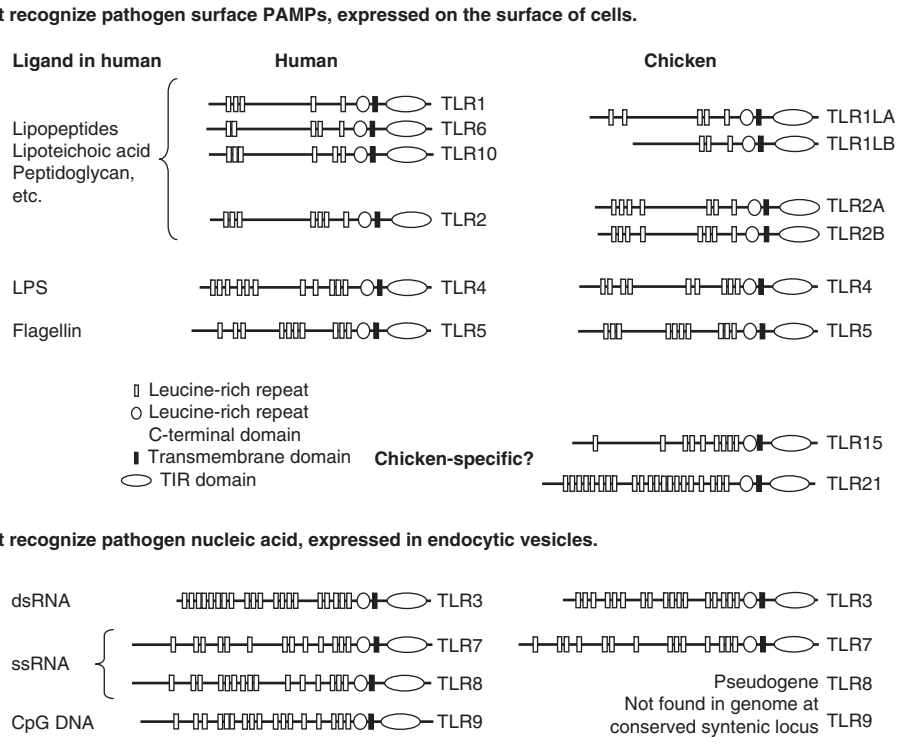

Fig. 2.1. A comparison of a major class of human and chicken PRRs, namely Toll-like receptors (TLRs). The natural ligands of the human TLRs are shown, and these are presumed to be the same ligands for the orthologous TLRs in the chicken. In human, TLR1 and TLR6 (and presumably TLR10) form functional heterodimers with TLR2. In the chicken, although there are only two TLR1/6/10 equivalents, TLR2 has been duplicated, and the two chicken TLR2 molecules can form functional heterodimers with the two chicken TLR1-like molecules (Keestra *et al.*, 2007; Higuchi *et al.*, 2008). Of the two chicken-specific TLRs, from the pattern of their extracellular leucine-rich repeats one would predict that chicken TLR15 recognizes a cell-surface PAMP, and that chicken TLR21 recognizes pathogen nucleic acid. It has recently been shown that chicken TLR21 recognizes Cp6 DNA (Brownlie *et al.*, 2009; Keestra *et al.*, 2010).

The best-characterized family of membrane-bound PRRs are the Toll-like receptors (TLRs), so called because of their evolutionary relationship to the *Drosophila* protein Toll, which is a member of a family of proteins in *Drosophila* that control dorsoventral patterning. Toll also has a role in defence against fungal infections in *Drosophila*, recognizing a fungal PAMP and triggering an intracellular signalling pathway leading to an innate response against the fungal infection. To date, 13 mammalian TLRs have been described, of which 11 are expressed in man. Birds and fish also express TLRs, some of which have functional homology with their mammalian counterparts, whereas others seem to be specific to birds (Roach *et al*., 2005; Higgs *et al*., 2006) or fish (Roach *et al*., 2005).

Toll-like receptors that recognize cell-surface components of pathogens are expressed on the host cell surface, whereas those that recognize pathogen nucleic acid are primarily expressed in endocytic vesicles (Fig. 2.1). Triggering of TLRs by their specific PAMPs can lead to the induction of several signalling pathways, including the NF-κB pathway, the MAP kinase pathways and the type I interferon (IFN) pathway, leading to the production of pro-inflammatory cytokines and chemokines and type I IFNs, and the induction of co-stimulatory molecules. This in turn leads to the recruitment and activation of other cells of both the innate and adaptive immune responses.

The major characterized family of cytoplasmic PRRs are the NOD-like receptors (NLRs; reviewed in Inohara *et al*., 2005), which regulate both inflammatory and apoptotic responses. NLRs are thought to oligomerize after recognizing their ligands, and thereafter activate inflammatory caspases, such as caspase-1, leading to the activation of pro-inflammatory cytokines such as IL-1β and IL-18 and/or to the activation of the NF-κB pathway.

There are two major subfamilies of NLRs: the NODs themselves and the NALPs (NAcht domain-, Leucine-rich repeat- and Pyrin-domain-containing proteins). Both NOD1 and NOD2 recognize peptidoglycan motifs of both Gram-negative (both) and Gram-positive (NOD2 only) bacteria. NODs interact with bacterial peptidoglycan via C-terminal leucine-rich repeats (LRRs) and signal via N-terminal caspase-recruitment domains (CARD) (Strober *et al*., 2006). NALPs are a large family of proteins (14 in man) that contain C-terminal LRRs, thought also to recognize PAMPs of bacterial pathogens, although their precise ligands are not fully understood.

A third group of cytoplasmic PRRs – RNA helicases – recognize viral double-stranded and single-stranded RNA. Three of these helicases have been described in mammals. RIG-I and MDA5 recognize 5′ triphosphate and dsRNA, respectively, activating antiviral signalling via twin N-terminal CARD domains. The third, LPG2, acts as a dominant-negative inhibitor.

Endocytic PRRs recognize carbohydrates on the surface of pathogens. They promote the recognition, uptake and destruction of pathogens by phagocytic cells, and do not trigger intracellular signalling pathways. Endocytic PRRs include the mannose receptor, a lectin-like recept'or present on APCs such as DCs and macrophages. Ligation of the mannose receptor triggers uptake of the pathogen via phagocytosis and endocytosis. Other endocytic PRRs, present on all phagocytic cells, include glucan receptors and scavenger receptors.

Other more classical molecules long known to have a role in innate immune responses can also be considered as soluble PRRs, such as the complement receptors, collectins, serum amyloid and C-reactive protein.

Pattern recognition receptors are obvious candidate genes for disease resistance. Single nucleotide polymorphisms (SNPs) have been identified in TLRs in several farm animal species, including the cow, pig and chicken (Leveque *et al*., 2003; Shinkai *et al*., 2006; Cargill and Womack, 2007; Seabury *et al*., 2007). Many of these SNPs are located in the ligand-binding LRR domains, thus raising the possibility of differential recognition of their ligands and hence differential downstream immune responses. For example, TLR4 polymorphisms have been shown to associate with resistance to respiratory syncytial virus infection in man (Puthothu *et al*., 2006), TLR2 and TLR4 polymorphisms with mastitis in sheep (Swiderek *et al*., 2006) and TLR4 polymorphisms with *Salmonella* resistance in chickens (Leveque *et al*., 2003).

Defensins

Defensins are small, cationic, anti-microbial peptides found in plants, insects, birds and mammals. They have two main functions, to bind to microbial cell membranes and form pores therein, thus directly leading to microbial cell death, and to chemoattract effector cells (e.g. macrophages and mast cells) of the innate immune response to help kill the microbe (Soruri *et al*., 2007). In mammals, there are three classes of defensins: α- and β-defensins are the main classes, produced by all mammals so far studied, with a smaller class, θ-defensins, produced only by non-human primates. In birds, by contrast, there is only a β-defensin family. α- and β-defensins are expressed primarily in leukocytes and epithelial cells, and are either constitutively expressed in granules of phagocytic cells such as neutrophils for immediate release or are inducible in response to signalling from PRRs.

Polymorphisms in defensin genes have been associated with disease resistance or susceptibility in a number of species. For example, defensin β-1 gene polymorphisms are associated with asthma in man (Levy *et al*., 2005). Polymorphisms in β-defensin 4 are associated with somatic cell counts in Holstein-Friesian cattle (Bagnicka *et al*., 2007), and polymorphisms in the chicken β-defensin gene cluster are associated with resistance to *Salmonella* infection (Hasenstein *et al*., 2006; Hasenstein and Lamont, 2007).

Neutrophils

Neutrophils and their avian equivalent, heterophils, are polymorphonuclear (PMN) cells (alongside eosinophils and basophils) that actively phagocytose invading pathogens. They are considered the main effector cells of an induced innate immune response, being the first cell type to respond in number (often within an hour) to the site of an infection, particularly bacterial infections, in response to chemokines. After internalizing pathogens into a phagosome,

neutrophils kill them by a variety of mechanisms. In the process known as the respiratory burst, NADPH oxidase is activated and produces a large quantity of the reactive oxygen species, superoxide. Superoxide in turn is converted spontaneously or via enzyme activity to hydrogen peroxide, which in turn is converted to hypochlorous acid, which is thought to have bacteriocidal activity. Neutrophils also kill pathogenic microbes by the release of proteins in granules into the phagosome, a process known as degranulation. These proteins include molecules such as cathelocidin, cathepsin, myeloperoxidase and defensins.

Neutrophils can also release a net of fibres made up of chromatin and serine proteases. These neutrophil extracellular traps (NETs) are thought to trap and kill microbial pathogens extracellularly, independent of phagocytosis, and may also act as a physical barrier to microbial spread.

The best-studied system that demonstrates that differential PMN function is associated with disease resistance is Mike Kogut's group work with the avian heterophil and two of Cobb-Vantress' commercial lines of chickens: line A is resistant to salmonellosis, and line B is susceptible. Increased *in vitro* heterophil function in line A (Swaggerty *et al*., 2003a,b) correlates with increased *in vivo* resistance to organ invasion by *Salmonella enteritidis* (Ferro *et al*., 2004; Swaggerty *et al*., 2005). Further, heterophils from the resistant line produce greater levels of pro-inflammatory cytokines and chemokines than heterophils from the susceptible line (Swaggerty *et al*., 2004). It is evident that there are clear measurable differences in heterophil function and innate immune responsiveness between these two lines, and that these are under specific genetic control and are sex-linked (Swaggerty *et al*., 2003a). By profiling the mRNA expression levels of pro-inflammatory cytokines and chemokines, Kogut's group have now identified sires from a broiler population with differential expression of these molecules, and from these sires have generated progeny with similar expression profiles; in other words, they have selected birds with increased innate immune responsiveness (Swaggerty *et al*., 2008).

γδ T cells

In biomedical model species, γδ T cells are a small subset of T cells bearing a distinct T cell receptor (TCR), TCR-γδ. They are found in highest abundance amongst intra-epithelial lymphocytes in the gut mucosa, suggesting that their primary role is in immune surveillance at this site. In farm animal species, however, γδ T cells are a far more prevalent subset, particularly in ruminants (Hein and Mackay, 1991) and chickens, where the peripheral T cell pool contains 20–50% γδ T cells (Cooper *et al*., 1991), compared with 5% in man and mouse.

The ligands for the TCR-γδ are not fully characterized, although they can recognize lipid antigens. The ligands do not seem to require antigen processing and are not presented in the context of classical MHC, though some may recognize antigens presented by non-classical MHC class Ib molecules, and others seem to recognize antigens directly.

Activation of γδ T cell responses is not fully understood. Mature γδ T cells can be divided into functionally distinct subsets, although the roles of these

subsets are poorly characterized. It is obvious, however, that they have multiple direct and indirect effects on both pathogens and those cells and tissues responding to those pathogens.

γδ T cells were considered as a first line of defence, but are now thought of more as a link between innate and adaptive immune responses (Holtmeier and Kabelitz, 2005). Since they rearrange their TCR genes and produce junctional diversity, they can be considered as part of the adaptive immune response. They also develop a memory phenotype. However, they can also be considered as part of the innate immune response, as their restricted TCR may be used as a PRR (Born *et al*., 2006; Morita *et al*., 2000), and they respond very rapidly to pathogens.

NK cells

Natural killer (NK) cells, so called because they are pre-programmed to kill certain types of target cells – particularly virus-infected cells or cancerous cells – are a small population (approximately 2%) of the cells in peripheral blood. NK cells express both activating and inhibitory receptors on their surface. The former activate the NK cells when they bind to a target cell. The latter, or killer inhibitory receptors (KIRs), transmit an inhibitory signal if they recognize MHC class I molecules on the cell surface. All normal cells express a certain level of MHC class I on their surface. MHC class I molecules present viral antigens in infected cells to the adaptive immune response, in particular CD8 cytotoxic T cells, which kill the virally infected cell (see below). As an immune evasion mechanism, many viruses encode proteins that downregulate the expression of MHC class I molecules on the cell surface to avoid CD8 T cell killing. However, as NK KIRs recognize MHC class I on the cell surface, lack of class I expression in a virally infected cell means that the inhibitory signal from the KIR is not transmitted to the NK cell, which only then receives an activating signal and as a result kills the infected cell.

Natural killer cells kill by releasing granules containing perforin and granzymes. Granzymes are serine proteases that induce apoptosis (Bots and Medema, 2006). Perforin was so named because it was thought to create pores within the target cell, allowing the granzymes to enter. However, it is now thought that a complex of proteins, including perforin and granzyme B, enters the target cell through the mannose 6-phosphate receptor, and becomes enclosed in a vesicle. Perforin then allows the granzyme B to exit the vesicle and cause apoptosis (Buzza and Bird, 2006). NK cells also express cytokines, and thus can influence local innate and subsequent adaptive responses.

Natural killer cell receptor genes are polymorphic and individual NK cells only express a subset of the available KIR genes (between 4 and 14 in humans), raising the possibility that KIR genes play a role in disease resistance. Promoter polymorphisms have recently been shown to regulate the frequency with which a particular KIR gene is expressed within an individual's NK cell population, and this may contribute substantially to phenotypic variation in disease resistance (Li *et al*., 2008).

NK T cells

Natural killer T cells are T cells in that they express an αβ TCR, albeit of limited repertoire; yet they also express some cell-surface markers characteristic of NK cells, hence their name (Godfrey *et al*., 2004). The restricted TCR-αβ repertoire responds to lipid and glycolipid antigens presented by the non-polymorphic CD1b molecule, rather than to peptide antigens presented by MHC molecules. NK T cells can express either IFN-γ or IL-4/IL-13, and are thus thought to provide rapid help to drive adaptive immune responses towards either cell-mediated or humoral. Again, they are thus thought to be a link between innate and adaptive immune responses.

Dendritic cells

Dendritic cells, first described as such by Steinman and Cohn (1973), are capable of activating naive T cells much more efficiently than B cells or macrophages, and thus are known as professional APCs. They also capture and process antigens more effectively than the other APCs, and express higher levels of MHC and co-stimulatory molecules on their surface once mature. In the periphery, they are classically defined as being in an immature, immune surveillance, antigen-uptake mode, highly phagocytic and endocytic, yet expressing low levels of co-stimulatory molecules and being poor stimulators of naive T cells. Once they have captured and processed antigens, in mammals they migrate to the local draining lymph node and mature to an antigen-presenting mode, losing the ability to phagocytose and endocytose, but upregulating co-stimulatory molecules and becoming potent stimulators of naive T cells. Non-mammalian species, however, lack lymph nodes and in these species the site of antigen presentation by DCs to the adaptive immune response is, to date, poorly understood.

In mammals, DCs can be broadly subdivided into myeloid DCs and plasmacytoid DCs, which differ in their expression of TLRs. Myeloid DCs mainly express TLR2 and TLR4, and predominantly express IL-12 once stimulated, thus driving cell-mediated adaptive immune responses. Plasmacytoid DCs mainly express TLR7 and TLR9 and produce high amounts of IFN-α on stimulation.

Most research has concentrated on the role of DCs in acting as the bridge between the innate and adaptive immune response, in driving appropriate adaptive immune responses to deal with the nature of the infectious pathogen. However, recently it has become apparent that DCs play an intimate role in the innate immune response itself, interacting with other innate immune response cells such as NK cells, NK T cells and γδ T cells (Andrews *et al*., 2005; Reschner *et al*., 2008). The crosstalk between DCs and the other innate lymphocytes is bidirectional and includes both cell–cell contact and soluble factors such as cytokines and chemokines. NK cells and NK T cells can also kill infected DCs. The final outcomes of these interactions can have considerable impact on the downstream responses to infection, in terms of both strength and specificity.

Macrophages

Macrophages share many of the functions of DCs. Indeed, there is currently a debate in biomedical model species as to whether macrophages and DCs are, in fact, distinct cell subsets, or if they should be considered as a single cell type. Macrophages play a role in both innate immunity and adaptive immunity. Like DCs, they phagocytose cellular debris and pathogens, present antigens to lymphocytes and other immune cells in the context of the MHC and express co-stimulatory cell-surface molecules, cytokines and chemokines to drive adaptive responses. However, distinct from DCs, macrophages cannot stimulate naive T cells to proliferate.

Acute phase response

The acute phase response is central to the innate host defence system against trauma, inflammation and infection (Petersen *et al*., 2004), and is composed of a wide range of systemic reactions including the production of acute phase proteins (APP) by the liver and increased secretion of the APP into the circulation. The production of APP is stimulated by the pro-inflammatory cytokines IL-1β, IL-6 and TNF-α, with the liver being the major site of APP synthesis. Many important diseases of animals are known to elevate the concentrations of APP (Murata *et al*., 2004; Petersen *et al*., 2004). Assays for APP are being used to monitor the health status of animals in experimental models of disease and potentially in health assessment programmes on the farm.

Adaptive Immunity

Adaptive immune responses are normally required to clear pathogens and generally lead to immunological memory, either as a result of primary infection with a pathogen or in response to vaccination. They can be broadly subdivided into two types of responses: those required to clear intracellular pathogens and those required to clear extracellular pathogens. Intracellular pathogens, such as viruses, intracellular bacteria and intracellular protozoan pathogens, are cleared by what have classically been known as cell-mediated adaptive immune responses. In biomedical model species these are now considered as inflammatory responses. Extracellular pathogens, such as extracellular bacteria, extracellular protozoan parasites and helminth worms, are cleared by humoral immune responses, with a major role for antibody, and eosinophilia. These responses in biomedical model species are now described as allergic and anti-helminthic worm responses. Nevertheless, for both types of responses the molecules and cells involved remain the same.

Cell-mediated and humoral responses are driven at least in part by two distinct subsets of cytokines produced, it is believed, by two distinct subsets of CD4+ helper T cells, Th1 and Th2, respectively. This paradigm was first coined two decades ago by Mosmann and Coffman (Mosmann *et al*., 1986; Mosmann

and Coffman, 1989) and has been central to our understanding of adaptive immune responses in biomedical model species. The paradigm has also been extended to include different antibody isotypes, CD8+ T cells, DCs, etc. However, the applicability of the paradigm to other mammalian species is less clear, at least in certain aspects. The paradigm was recently shown, in its original definition, to extend beyond mammals to the chicken (Degen *et al.*, 2005), in that cytokine responses to an intracellular pathogen were predominated by IFN-γ (a Th1 cytokine) and those to an extracellular pathogen by IL-4 and IL-13 (Th2 cytokines).

Since the paradigm was first proposed, it has become clear that there are more than two CD4+ T cell subsets, with either regulatory or effector functions, and these will be described in more detail below.

As already described earlier, adaptive immune responses are triggered when pathogen antigen is presented by APCs to T cells, which recognize that peptide antigen in the context of MHC on the APC. As a result, either CD4+ or CD8+ T cells are stimulated to proliferate and become activated. CD8+ T cells are cytotoxic and kill infected cells directly. CD4+ T cells, as mentioned above, have effector or regulatory phenotypes. The effector functions include providing help to cytotoxic T cells, NK cells, macrophages and other effector cells of the cell-mediated immune response (if Th1 cells) or to eosinophils and B cells (if Th2 cells). Regulatory functions are mainly involved in dampening down inflammatory Th1 responses. The end result is, of course, clearance of the pathogen and the establishment of immunological memory.

MHC

MHC proteins display both self and non-self antigens on the surface of the cell. For the purposes of this chapter, we shall discuss the presentation of non-self, or pathogen, antigen. MHC class I presents endogenous peptide (i.e. generally from intracellular pathogens) to CD8+ cytotoxic T cells, which are then activated to recognize and kill infected cells presenting the same peptides in the context of their MHC class I. MHC class II presents exogenous peptide (i.e. generally from extracellular pathogens) to CD4+ T cells, which differentiate into T cell subsets with either effector or regulatory phenotypes, as described in detail later.

MHC class I is expressed on the surface of virtually every cell. MHC class I molecules are heterodimers consisting of a single transmembrane α-chain and β2-microglobulin. The α-chain has three polymorphic domains – α1, α2 and α3 – α1 and α2 forming the peptide-binding groove of the molecule. Peptides bound by MHC class I are generally 8–9 amino acids in length. The peptide-binding groove often has deep pockets that bind 'anchor' residues in the peptide. The peptides bound by MHC class I are generated in the cytosol by the proteasome, which breaks proteins into peptides that are then transported into the endoplasmic reticulum (ER) by the two transporters associated with antigen-processing (TAP) molecules. The MHC class I molecules are then loaded with the peptides in the lumen of the ER in a process that includes several other

molecules including calnexin, calreticulin, ERP57 and tapasin. MHC class I loaded with peptide then passes through the normal secretory pathway from the ER to the cell surface, where it can present bound peptide to T cells.

The expression of MHC class II molecules is more restricted, primarily to the surface of APCs. MHC class II molecules are also heterodimers, consisting of α- and β-chains. The open-ended peptide-binding groove is formed by the α1 and β1 domains of the two chains, and can bind peptides of 15–24 amino acids. Peptides bound by MHC class II are derived from extracellular proteins which are endocytosed, chopped up in lysosomes and bound to MHC class II as the latter migrates to the cell surface.

In mammals, the MHC is a large, complex genetic region with many highly expressed classical class I and class II genes (Trowsdale, 1995; MHC Sequencing Consortium, 1999). In man, the MHC is encoded on chromosome 6 in a 3.6 Mb region encompassing 140 genes (MHC Sequencing Consortium, 1999). The MHC region is divided into three subgroups: class I and class II, separated by class III. The picture may be different in non-mammalian vertebrates. For example, the chicken has a 'minimal essential MHC', encompassing 92 kb and 19 genes, with single dominantly expressed class I and class II genes (two of each are present in the chicken MHC region) in most common MHC haplotypes (Kaufman *et al*., 1999a,b). The class I and II 'regions' in the chicken are side by side in the genome, rather than separated by the class III region. In contrast to the two class I genes present in the chicken MHC, the duck MHC contains five class I genes (Moon *et al*., 2005). Despite this, the duck MHC class I region functionally resembles the minimal MHC of the domestic chicken in that only one of these five genes is dominantly expressed (Moon *et al*., 2005).

In mammals, there is only limited evidence that MHC genes confer enhanced resistance against infection and that this can be selected for. Despite the fact that the high polymorphism of mammalian MHC genes is thought to have been driven by pathogen challenge, the different haplotypes show little consistent pattern of resistance against most pathogens. The strongest associations of mammalian MHC are with autoimmune diseases, and those with infectious disease are limited (Tiwari and Terasaki, 1985; Hill, 1998). However, disease resistance associated with the MHC has been demonstrated with high somatic cell counts in milk induced by infection (Dietz *et al*., 1997) and bovine leukaemia virus (Lewin and Bernoco, 1986) in cattle.

There are far stronger associations between the MHC and infectious disease resistance in the chicken. The presence of a single dominantly expressed class I gene might at first glance seem a suicidal strategy. That particular MHC either binds a peptide from a pathogen, thus stimulating an immune response that may enable the bird to survive, or fails to bind a peptide, in which case no immune response is stimulated and the bird may die. Indeed, the elegant work of Wallny *et al*. (2006) demonstrates that this is indeed the case for responses of chicken haplotypes to infection with Rous sarcoma virus, a pathogen that only encodes four proteins.

Another viral disease of chickens with very strong MHC association is Marek's disease (MD), caused by the herpesvirus MD virus (MDV). MDV

encodes more than 80 proteins, and thus it is likely that any chicken MHC haplotype would find an MDV peptide to bind. However, resistance to tumours caused by classical MDV in the B21 haplotype is one of the strongest associations in any species between disease and the MHC (e.g. Calnek, 1985; Plachy *et al*, 1992; Kaufman and Lamont, 1996; Kaufman, 2000). Resistance to MDV has been studied in many different haplotypes, which can be ranked by their relative resistance/susceptibility. This rank order correlates with the relative level of class I molecules expressed on the cell surface (Kaufman *et al*., 1995; Kaufman and Salomonsen, 1997). This can vary by tenfold or more between chicken haplotypes, but is remarkably consistent between mammalian haplotypes (although it varies considerably between cell types). Surprisingly and perhaps counter-intuitively, the most resistant haplotype, B21, has the lowest levels of MHC class I on the cell surface (Kaufman, 2000). The precise mechanism underlying this resistance is, however, still unclear.

T cells

NK T cells and γδ T cells have already been described. This section will therefore concentrate on the two TCR-αβ-bearing T cell subsets, CD4 and CD8. CD4 and CD8 are co-receptors for the TCR and assist in activation of the T cells bearing these molecules following interaction with an APC, by recruiting tyrosine kinases to help trigger the resulting signalling cascade. CD4 and CD8 also interact directly with the MHC class II and class I molecules, respectively, on the APC interacting with the TCR on the T cell.

CD4+ T cell subsets

For many years there were considered to be only two subsets of CD4+ T cells: Th1 and Th2. A number of CD4+ T cell subsets with regulatory function were then described, followed by another subset with effector phenotype, Th17. Recently another effector subset, Th9, has also been postulated. Figure 2.2 summarizes these subsets, the cell-surface markers and cytokines they express, and the transcription factors that drive their differentiation and subsequent cytokine profiles, at our current level of understanding in biomedical model species. However, the degree to which these cellular subsets and their associated molecules apply to farm animal species remains to be established. For example, IL-4 does not appear to be central to the Th1/Th2 paradigm in pigs (Murtaugh *et al*., 2009), and IL-13 is preferentially expressed over IL-4 in Th2 responses in the chicken (P. Kaiser, unpublished).

Effector CD4+ T cells

Th1 cells are currently thought to differentiate from proliferating precursor CD4+ T cells under the influence, among other things, of cytokines such as IL-12 and IL-18. The signature cytokine produced by Th1 cells, driven by the transcription factor Tbet (Szabo *et al*., 2000), is IFN-γ, which in turn drives

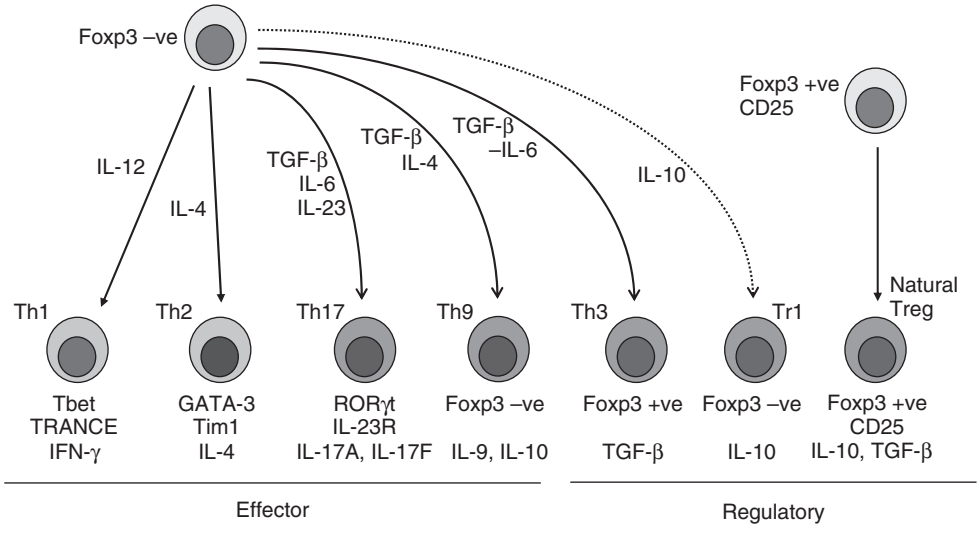

Fig. 2.2. CD4+ T cell subsets in biomedical species. These can be subdivided into those with effector functions and those with regulatory functions. The cells differentiate either from a Foxp3 –ve proliferating precursor CD4+ T cell or, in the case of natural Treg cells, from a naive Foxp3 +ve CD4+ T cell expressing CD25. Cytokines influencing the differentiation events are shown next to the arrows linking the precursor to the relevant subset. For example, TH17 cells are driven by TGF-β, IL-6 and IL-23, whereas Th3 cells are driven by TGF-β in the absence of IL-6. Underneath the subsets are listed transcription factors, cell-surface molecules and signature cytokines that act as markers of these subsets. For example, Th1 cells express the transcription factor Tbet, the TNF superfamily member TRANCE on their cell surface, and express the signature cytokine IFN-γ.

cell-mediated adaptive immune responses. Th1 cells, in biomedical model species, can also be distinguished by the expression of the chemokine receptor CCR5 (Ødum *et al*., 2002), but not CCR3, and the expression of two other cell-surface molecules – TRANCE (a member of the TNF superfamily, also known as RANK ligand, or RANKL) and Tim-3 (Josien *et al*., 1999; Monney *et al*., 2002).

Th2 cells, similarly to Th1 cells, differentiate from proliferating precursor CD4+ T cells, but this is thought to be driven, in the absence of IL-12, by IL-4. The signature cytokines produced by Th2 cells, driven by the transcription factor GATA-3 (reviewed in Zhu *et al*., 2006), are IL-4 and IL-13, which in turn drive humoral immune responses. Like Th1 cells, in biomedical model species Th2 cells can also be distinguished by the expression of chemokine receptors. Th2 cells express CCR3 and CCR7 (Sallusto *et al*., 1997; Zingoni *et al*., 1998), but not CCR5. They also express another cell-surface molecule, Tim-1 (de Souza *et al*., 2005; Meyers *et al*., 2005).

Th17 cells are a newly described subset of Th cells (Harrington *et al*., 2005; Steinman, 2007; Stockinger and Veldhoen, 2007) that produce two members of the IL-17 family – IL-17A and IL-17F – which are involved in the recruitment and activation of neutrophils. They also produce IL-21 and IL-22

(Ouyang *et al*., 2008). In mammals, Th17 cells have mainly been studied in autoimmune disease, particularly autoimmune inflammation (Ouyang *et al*., 2008). However, they probably also play a role in responses to infection with certain bacteria and fungi (Zelante *et al*., 2007), during which IL-17 is preferentially induced.

The mechanisms by which proliferating precursor CD4+ T cells are differentiated into Th17 cells is unclear. In man and mouse, TGF-β, IL-6, IL-21 and IL-23 have been shown to play a role (Dong, 2008; Manel *et al*., 2008), as has the transcription factor RORγt (Ivanov *et al*., 2006). IL-23 is thought to expand established populations of TH17 cells, but cannot induce their differentiation from naive precursors (Bettelli *et al*., 2006). IL-21 may well have an autocrine effect, as it can also activate Th17 cells (Korn *et al*., 2007). Th17 differentiation is negatively regulated by both IFN-γ and IL-4, suggesting that either a Th1 or Th2 response will switch off a Th17 response.

Th9 cells are the most recently described CD4+ T cell subset (Dardalhon *et al*., 2008; Veldhoen *et al*., 2008). TGF-β, which plays an important role in the differentiation of Th17 cells (see above), and also in the differentiation of inducible regulatory CD4+ T cells (see below), can reprogram Th2 cells to switch to IL-9 secretion (Veldhoen *et al*., 2008). In combination with IL-4, TGF-β can directly drive the differentiation of Th9 cells from naive cells (Veldhoen *et al*., 2008). A similar population of cells generated with TGF-β and IL-4 were reported by Dardalhon *et al*. (2008) as Foxp3$^-$ (Foxp3 is the signature transcription factor of regulatory CD4+ T cells – see below), but expressing IL-9 and IL-10 (IL-10 is a major regulatory cytokine – see below). Despite this, they have no obvious regulatory activity but instead seem to promote tissue inflammation (Dardalhon *et al*., 2008).

Regulatory CD4+ T cells

Evidence has existed for several decades of CD4+ T cells that suppress immune responses, particularly pro-inflammatory immune responses but also humoral responses. These cells used to be termed 'suppressor' T cells, but were difficult to study because of difficulties in isolating clonal lines. However, the past 6 or 7 years have seen a resurgence in the interest in 'suppressor' or regulatory CD4+ T cells, which are now considered to fall into up to three subsets: natural Treg cells, Tr1 cells and Th3 cells.

Natural Treg cells comprise about 10% of peripheral CD4+ T cells. They express CD25, the α-chain of the IL-2 receptor, as naive cells (CD25 is normally only expressed on other CD4+ T cells after activation) (Apostolou *et al*., 2002; Shevach, 2002). They also express the transcription factor Foxp3 (Gavin and Rudensky, 2003) and the cell-surface marker CTLA-4. On activation via the recognition of antigen presented in the context of MHC class II on APCs, and co-stimulation from B7 molecules (CD80 and CD86), natural Treg cells rapidly express large quantities of IL-10, which is inhibitory for the production of pro-inflammatory (Th1) cytokines such as IFN-γ. CTLA-4 on the surface of natural Tregs binds tightly to the B7 molecules on the APCs, and probably in one sense sequesters these molecules so that they are not available

to provide co-stimulation to effector CD4+ T cells, thus causing further suppression of effector responses.

The other two regulatory subsets differentiate following activation. Both subsets are abundant in the intestine of biomedical model species.

Tr1 cells do not express CD25 on their surface when naive, nor do they express Foxp3. However, they require IL-10 for their differentiation and secrete large amounts of IL-10 and TGF-β, another cytokine with anti-inflammatory properties (Asseman and Powrie, 1998).

Th3 cells differentiate in the presence of TGF-β and the absence of IL-6, and express Foxp3 and TGF-β, but not IL-10 (Chen *et al.*, 2003).

CD8+ T cells

CD8 occurs in two isoforms, α and β, and is either an α-homodimer or an αβ-heterodimer. CD8-bearing T cells are also known as cytotoxic T cells (Tc) since they directly destroy cells infected with intracellular pathogens, particularly viruses, and tumour cells.

Activation of CD8+ T cells requires recognition of antigen presented in the context of MHC class I by an APC and co-stimulation between CD80/86 on the APC and CD28 on the CD8+ T cell. Like NK cells (see above), following activation CD8+ T cells release perforin and granulysin, leading to apoptosis of the infected cell. Activated CD8+ T cells can also trigger apoptosis through direct cell–cell contact with the infected cell. Activation upregulates expression of Fas ligand (FasL) on the surface of CD8+ T cells, which can then interact with Fas on the infected cell, triggering signalling pathways and leading to apoptosis of the infected cell.

CD8α/α homodimer-expressing cells are found predominantly in the gut on the surface of many intra-epithelial lymphocytes. These CD8α/α homodimers interact specifically with a non-classical MHC molecule, TL, expressed on intestinal epithelial cells (Leishman *et al.*, 2001). It is now apparent that only CD8α/β heterodimers act as co-receptors for the CD8-dependent TCR (McNicol *et al.*, 2007).

B cells and antibody

B cells mature in the bone marrow in mammals and in the bursa of Fabricius in birds (hence their name – B for 'bursa'). B cells produce antibody (or immunoglobulin (Ig)) against pathogen antigen as part of the humoral adaptive immune response. They can act as APC. They can also develop into memory B cells with appropriate stimulation. B cells recognize protein epitopes, which can be either linear or conformational, in their native form either as soluble proteins or on the surface of cells or pathogens. This recognition is achieved via the B cell receptor (BCR), which is essentially membrane-bound or surface Ig (mIg or sIg). Each B cell expresses a unique BCR that will recognize one particular epitope on a particular antigen, and a typical human B cell will have up to

100,000 of these clonal BCRs on its surface. Binding of this cognate antigen, provided the B cell receives an additional signal, or 'help' from a Th2 CD4+ T cell, leads to activation of the B cell and its differentiation into either a plasma B cell, producing large amounts of antibody, or a memory B cell. This differentiation can occur directly or after additional steps (including somatic hypermutation and potentially isotype switching – see below) in a germinal centre.

B cells can be divided into subsets, like T cells. As described above, plasma B cells produce large amounts of Ig. They are short-lived cells that undergo rapid apoptosis once the pathogen stimulating the immune response has been cleared, as they are no longer exposed to the cytokines and other factors necessary to promote their survival. Memory B cells are long-lived and respond very quickly to produce large amounts of Ig, often with higher affinity, following re-exposure to the same antigen. B cells are also divided into B-1 and B-2 cells. The latter are conventional B cells, whereas the BCRs of B-1 cells have low affinity for many different antigens and these cells express IgM in greater quantities than IgG. Their BCRs have a preference for other Ig, self-antigens and common bacterial polysaccharides. They are found predominantly in the peritoneal and pleural cavities and belong to a distinct developmental lineage to the B-2 cells (Montecino-Rodriguez and Dorshkind, 2006).

Naive B cells, i.e. those that have not been exposed to antigen, can be activated via a T cell-dependent or -independent mechanism. Most antigens are T dependent and this mechanism involves direct interactions between Th2 cells and the B cell, as described earlier, via T cell TCR/B cell MHC-expressing-peptide interactions, co-stimulatory interactions such as between CD40L on the Th2 cell and CD40 on the B cell, and the action of cytokines such as IL-4, IL-5 and IL-13 produced by the Th2 cell. Memory cells and isotype switching are induced in T-dependent responses.

A substantial number of antigens are T independent, however, and deliver sufficient stimulatory signals, by cross-linking BCRs, to activate B cells in the absence of T cell help. This can either be via the binding to several BCRs of repeat carbohydrate units on the surface of, for example, bacteria (type 1) or via the presentation by macrophages of multiple copies of the same pathogen antigen in such a way that the BCRs are cross-linked (type 2).

Ig typically consists of two heavy chains and two light chains. Camels and llamas are two exceptions – in these species Ig consists of only paired heavy chains (Muyldermans, 2001). In mammals, five isotypes of Ig are known: IgD, IgM, IgG, IgA and IgE. In lower vertebrates, this number of isotypes is variable. For example, birds and fish have only three, but each has a different repertoire. Birds have IgM, IgY (the functional equivalent of IgG) and IgA, whereas bony fish have IgD, IgM and IgT/IgZ (which seems to be specific to bony fish – Danilova *et al*., 2005; Hansen *et al*., 2005) and cartilaginous fish have IgM, IgW/IgX/IgNARC and IgNAR (with the latter two specific for cartilaginous fish – Dooley and Flajnik, 2006). IgG (and in some cases IgA) in mammals can be subdivided into different subclasses, the number and nomenclature of which vary from species to species. Certain subclasses predominate during Th1 responses and tend to be opsonizing Ig, whereas other subclasses predominate during Th2 responses and tend to be neutralizing Ig.

Both heavy and light chains consist of variable (V) and constant (C) domains – one of each for the light chain, and 1 V and 3/4 C for the heavy chain. The variable domains of both chains together contain the site that binds antigen. Each V region contains three variable loops, or complementarity-determining regions (CDRs), that form the antigen-binding site. The mechanisms by which variation is introduced into these CDRs are discussed below. The last 2/3 C domains of the heavy chains make up the Fc region of Ig, and interact with specific Fc receptors on effector cells to mediate some Ig functions (see below).

In mammals, immature naive B cells express only IgM on their surface, but then express IgD also when they reach maturity. After engagement of the BCR with antigen, the B cell becomes activated, proliferates and can differentiate into a plasma cell producing antibody in secreted form. Isotype switching to IgG (and class switching), IgA or IgE can occur, triggered and controlled by cytokines.

Igs have several main functions. They bind to pathogens, preventing them from entering and/or damaging cells. They can opsonize a pathogen, stimulating its uptake by PMN cells such as macrophages or neutrophils, leading to its subsequent destruction. They can also stimulate pathogen destruction directly by activating complement. Cells recognize Ig via isotype-specific Fc receptors that interact with the constant regions of the Ig heavy chains. Receptor interaction triggers the cell's effector function. Fcγ receptors are widely expressed on PMN cells and induce phagocytosis of Ig-opsonized pathogens. The single Fcα receptor is also widely expressed and triggers similar functions. The high-affinity Fcε receptor is expressed on mast cells, eosinophils, basophils and Langerhans cells, and plays a major role in controlling allergic responses and triggers degranulation.

Ig diversity is controlled by several mechanisms: domain variability, V(D)J recombination, somatic hypermutation, affinity maturation and class switching. The first two mechanisms involve diversity generated by the recombination of different V(D)J segments in the light and heavy chain genes. First, the individual segments recombined will differ from chain to chain. Second, this process is not perfect, and nucleotides can be deleted or added as the recombination occurs, leading to increased variability. Once B cells are activated, they proliferate rapidly. In these rapidly dividing cells, the rearranged genes encoding the Ig chains undergo a high rate of point mutation (or somatic hypermutation), which introduces further variability. It can also alter the antigen-binding affinity of the Ig, in either direction. Those B cells that express higher-affinity Ig will receive stronger co-stimulatory signals from Th cells they interact with (affinity maturation). Class switching has been described above.

Breeding for Disease Resistance at the Level of the Immune Response

The adaptive immune response is designed to be absolutely specific against the pathogen causing the infection. For example, initial infection or vaccination with one species of *Eimeria* in chickens provides complete protection against subsequent challenge, but that protection is almost completely species-specific

(Rose, 1973). Further, control of these responses for effective clearance and memory is tight. Different responses are triggered to control intracellular (Th1) and extracellular (Th2) pathogens. Th1 responses downregulate Th2 responses, and vice versa. We are only now beginning to understand the complexity of CD4+ T cell subsets and the role they play in controlling adaptive immune responses. Selection for resistance at the level of an adaptive response is possible, but potentially problematic. It is unlikely to give resistance to even closely related pathogens and, depending on the gene(s) involved, it is possible that it might lead to increased susceptibility to some other pathogens.

The innate immune response, by contrast, is focused on responses to wide classes of pathogens. It also drives the subsequent (hopefully) relevant adaptive immune response. Selection for increased innate immune responses (or improved innate robustness) does therefore have potential to give greater resistance to a wider spectrum of pathogens, and is less likely to have deleterious effects.

Control of infectious disease in livestock species will continue to involve an integrated approach between genetic selection, biosecurity, pharmaceutical intervention (although this is likely to decrease) and vaccination. Exciting progress is also being made in understanding the interface between immunology, endocrinology and nutrition, and between probiotic bacteria, pathogenic bacteria, nutrition and the immune response. It should not be forgotten that the tools that enable us to dissect the immune response in farm animal species, and to detect markers and genes associated with disease resistance, can also be used to dissect the genetics of vaccine response.

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3 Modelling Farm Animal Diseases

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Summary

The modelling of diseases in domestic animal populations is often required to understand the complex interactions between the pathogen or parasite and the host animal, and to help to devise strategies to control or minimize the impact of the disease. A wide variety of approaches are taken to model diseases, including mathematical models derived using differential equations, simulation and transition-type matrices. These models may be formulated using both stochastic and deterministic settings. Models exist for a wide variety of diseases, and those which incorporate host genetics include a variety of microparasitic (e.g. viral and bacterial) and macroparasitic (e.g. nematode) infections in cattle, sheep and pigs.

The current state of knowledge regarding the joint modelling of host genotype and disease epidemiology is considered in this chapter. General mathematical frameworks are a requirement for these models, which can then be parameterized for specific diseases. It is suggested that for microparasitic infections the basic reproductive ratio, defined as a trait of the host population, is a means of linking host genotype and disease epidemiology, whereas for macroparasitic infections the disease biology has to be more explicitly modelled. Results are described for microparasitic diseases in which selection for increased resistance is expected to decrease the probability of epidemics as well as their severity. Appropriate methods for utilizing resistance genes are also discussed. For nematode infections, models that capture both the direct and indirect benefits of selection are described.

Ideally, epidemic models that include host genetics should be developed so that they can be used to support decision making in disease control. Further, they should enable animal breeders to prioritize diseases for research, and also correct weightings to be applied to disease and performance traits in breeding programmes. Future areas for research include development of epidemic models combining both host and parasite genetics, so that long-term sustainability of breeding for disease resistance can be assessed.

Introduction

This chapter describes the modelling of diseases in domestic livestock populations and the importance of such modelling when attempting to quantify the

benefits of selection for resistance to an infectious disease. Mostly these are epidemic models that are designed to capture the transmission dynamics of an infectious disease.

Why is it necessary to take an epidemiological modelling approach to address selection for disease resistance? A fundamental difference exists between the genetic improvement of productivity traits and the genetic improvement of traits describing resistance to infectious diseases. For production traits, animals express their genetic merit independently of each other, and benefits from selection can simply be summed across the population. However, for disease resistance there is often an interaction between animals in the expression of the trait. In simple terms, animals infect each other and the expression of the disease-resistance trait is dependent on the epidemiology of the disease itself. Thus, an interaction exists between the average host genotype and the disease epidemiology: transmission of infection between animals depends on host genotype, but the expression of the host resistance genotype depends on the prevailing force of infection. If this interaction is not accounted for, incorrect inferences may be made regarding the benefits of selecting for resistance.

This chapter briefly reviews the role of epidemiological modelling in understanding disease processes and the modelling approaches available. It then considers, in more detail, cases where host genotype for resistance has been considered as a factor in epidemic models and shows how genotype-by-epidemiology interactions may be quantified. It also considers the incorporation of the genetics of the pathogen into the model, as well as developments that may improve modelling of the host genotype effects on disease epidemiology and the use of such models in animal breeding.

The Attractions of Modelling

Describing and quantifying the processes and impacts of infectious diseases in domestic farmed animals presents a considerable challenge. Complex interactions occur between the host animal population and the pathogen, and these interactions will vary temporally, spatially and with the host genotype. Moreover, the infection and subsequent host immune responses are multifactorial. In order to predict the effect of any factor on an infectious disease at the population level, including treatment strategies or genetic selection of resistant hosts, an epidemiological model is required. This model should capture and quantify the dynamics of the infection or disease and allow the impact of various factors affecting the disease severity and transmission of infection to be predicted.

Major uses of epidemiological models have traditionally included: (i) evaluating the effects of various control strategies, e.g. vaccination or management options, on the spread of disease and the severity of epidemics; (ii) evaluating the impact of environmental factors such as local weather conditions on the disease severity; and (iii) evaluating the impact of various chemotherapy strategies on the likely evolution of drug resistance in the pathogen. Epidemiological models are now routinely used in the formulation of disease control policy, for example determining risks of exotic disease outbreaks and planning contingency measures

in cases where such outbreaks do occur. Such models are also used to assist in fire fighting in cases where epidemics such as foot-and-mouth disease occur.

This chapter considers the use of disease models, particularly epidemic models, for predicting the effects of genetic change in the host on the epidemiology of the disease. This is an area that has hitherto received less attention than non-genetic interventions to control disease; however, it is gaining increasing importance within the context of farm animal production.

Definitions and Modelling Approaches

A wide-ranging background to disease modelling is given by Anderson and May (1992). The application of mathematics to the study of infectious diseases was pioneered by David Bernoulli in 1760. He evaluated the effectiveness of variolation against smallpox, hoping to influence public health policy. In 1840 William Farr fitted a normal curve to smoothed quarterly data on smallpox deaths. Hamer (1906) postulated that the course of epidemics depends on the contact rate between susceptible and infected individuals, one of the most important concepts in epidemiology. This is the 'mass action' principle where the net rate of spread of infection is assumed to be proportional to the density of susceptibles multiplied by the density of infectives. In 1908 Ronald Ross translated the problem from a discrete-time model to a continuous-time framework in his work on the dynamics of malaria. Subsequently, much of the theory used to develop epidemic models has been based on differential calculus used to describe *rates* of transmission or processes, or based on stochastic approximations to such equations.

It is necessary, when modelling diseases, to consider both the modelling approach and the type of pathogen being modelled. Models are generally formulated either deterministically or stochastically, although there is overlap between these categories. Likewise, the modelling approach may be based on differential equations, simulation or transition-type matrices. Finally, the infection being modelled may be classified into those caused by microparasites, e.g. bacteria or viruses, or macroparasites, e.g. ticks or helminths. These classifications may be considered cross-classified, e.g. one can use either deterministic or stochastic formulations when modelling either macro- or microparasitic infections. A brief summary of these concepts is now given.

Pathogen type

Microparasites

Most viral and bacterial parasites, and many protozoan and fungal parasites, may be classified as microparasites. Microparasites may be thought of as those that have direct reproduction, usually at very high rates, within the host (Anderson and May, 1992). They tend to be characterized by small size, a short generation time which is often just a fraction of that of their host and a short duration of infection. Hosts that recover from infection usually acquire immunity against reinfection for some time, and often for life.

Unlike macroparasites such as nematodes, microparasites seldom have a closed life cycle that can be, or even requires to be, modelled. For such pathogens, the host population is often simply divided into a small number of classes of individuals, e.g. susceptible, infected, recovered or removed (leading to SIR models discussed below). An operational definition of a microparasite is an organism whose population biology can be sensibly described by such compartmental models (Anderson and May, 1992). Due to the rapid proliferation of the parasite once it is established, little or no account is generally taken of the degree of severity of infection, e.g. the abundance of the parasite within the host. Animals are considered to be either infected or not. The reality of infected animals with differing nutritional, environmental or genetic status is replaced by simple concepts of infected, immune, latent but not yet infectious, etc.

Macroparasites

Most helminths and arthropods may be classified as macroparasites. Macroparasites may be thought of as those not having direct reproduction within the host, i.e. having a part of their life cycle external to the host (Anderson and May, 1992). Typically they are larger and have longer generation intervals than microparasites, with the generation interval often being an appreciable proportion of the host's lifespan. Macroparasitic infections are typically of a persistent nature, with hosts being continually reinfected. The classic examples in domestic livestock are nematode infections of cattle, sheep and goats.

For macroparasitic infections, various factors describing the interaction between the host and the pathogen must be described when attempting to model the infection, e.g. egg output per parasitic female, pathogenic effects on the host, immune response and parasitic death rates. Thus, the simple compartmental models that are adequate for microparasitic infections must be replaced by more sophisticated models, which take account of the distribution of parasites among the host. An operational definition of a macroparasite is an organism whose population biology requires a full description of the distribution of parasites among hosts (Anderson and May, 1992).

Macroparasites are rarely distributed in a random way among their hosts. Typically they show an aggregated or clumped distribution, with the minority of hosts harbouring the majority of the pathogens. The distribution of parasites among hosts often conforms to a negative binomial distribution. The 80:20 rule is a useful approximation to such distributions: often 80%, or more, of the macroparasites will be contained in 20%, or fewer, of the hosts (Anderson and May, 1992). Pareto (1906) observed that the same rule of thumb applies to the distribution of wealth in human populations, a salient observation in the current climate of financial meltdown.

Model formulation

The distinction between deterministic and stochastic models is familiar to animal breeders. Deterministic models generally treat the values of input parameters as fixed, and produce point estimates of an outcome. Stochastic

models incorporate random variation in processes or parameters, and produce probability distributions of outcomes. In disease modelling, stochastic models are particularly appropriate for modelling the random contacts between susceptible and infected animals, e.g. as a time-dependent binomial distribution, hence producing a probability distribution of numbers of infected animals.

Modelling approach

Differential equations

The disease epidemic may be described by rates of change in, for example, the numbers of susceptible or infected animals, or the numbers of parasites for macroparasitic infections. Hence the essence of the process can often be captured in a few simple differential equations. For example, the rate of change in the number of adult macroparasites in a single host at time t, $dM(t)/dt$, when the host is constantly exposed to infection at a rate δ and the adult parasite dies at constant per capita rate μ, may be described as follows:

$$dM(t)/dt = \delta - \mu M(t)$$

with solution:

$$M(t) = M^*[1 - \exp(-\mu t)]$$

where M^* is an equilibrium population.

Extending such an approach to describing the parasite life cycle is conceptually simple. For example, as a first approximation, a ruminant nematode infection can be described by two differential equations: the first describing the change in host–parasite burden with time; and the second describing the change in pasture larval contamination with time. The equations may include terms describing the uptake of larvae by the host, the establishment probabilities of the parasite, worm burdens in the host, worm fecundity, host immunity, parasite survival probabilities, the density of the hosts on the pasture and the density of the larvae on the pasture. Roberts and Grenfell (1991) included a third equation describing changes in host immunity. Although this approach is deterministic in structure, it can nevertheless contain probabilistic elements to describe the distribution of parasite numbers per host, in the case of macroparasites.

For microparasitic infections, so-called compartmental models, where individuals are classified into discrete categories according to their infection status, are often used. For example, in the classic SIR model, the population of N animals may be classified as comprising S susceptible, I infected (infectious) and R recovered or removed animals. The model parameters are the transmission coefficient β and the recovery rate γ. Therefore, the following differential equations describe the dynamics of an epidemic:

$$dS/dt = -\beta SI$$
$$dI/dt = \beta SI - \gamma I$$
$$dR/dt = \gamma I$$

The use of differential equations to capture the essence of the epidemic is widespread in epidemiological modelling, due to its flexibility and relative simplicity. The procedure is usually a simplified case of the more complete approach using fully stochastic models. However, such stochastic models are often somewhat intractable to analytical investigation owing to the many non-linearities that are inherent in biological problems.

Simulation

Simulation models take many forms, with the main aim being to describe the disease patterns in relation to events that may change either deterministically or stochastically. Inputs into the simulation models may be explanatory variables describing the infection process, developed in the same way as the inputs into the mathematical differential-equations approach, or simply empirical relationships between observed phenomena – sometimes with no strong theoretical justification.

Classic applications of models using empirical relationships are the weather-based models describing helminth infection in cattle (Gettinby and Gardiner, 1980) and sheep (Barnes *et al*., 1988). In these models, the parasite epidemiology is described as a function of the prevailing weather conditions in any particular season, and inputs to the model include a database of meteorological records over many years.

Many simulation models include a combination of empirical and explanatory relationships, couched in both deterministic and stochastic terms. Such an example is the model of Bishop and Stear (1997) investigating the inheritance of resistance to nematode parasites in sheep. In their model, the life cycle of the parasite is defined in explanatory deterministic terms, empirical relationships are used to describe the development of host immunity and stochastic between-animal variation is used to describe the variability in the host–parasite interactions at the population level. Not all disease simulation models consider epidemiological effects. For example, the model of Vagenas *et al*. (2007a,b) considers the mechanisms underlying the interaction between a host sheep and nematode parasites; however, it ignores population-level impacts of infection.

Finally, fully stochastic models may be formulated using Markov-chain processes, in the cases where analytical solutions to the deterministic or stochastic models are intractable. This is particularly appropriate for microparasitic infections. Not only will this technique supply probability densities for various outcomes, it may even supply answers that are qualitatively different from simpler deterministic models. For example, as well as describing 'major epidemics' where most of the population is infected, such simulation models will also give rise to 'minor epidemics' where there is a probability of the disease arising in a population, then dying out through chance events (MacKenzie and Bishop, 2001a, b).

Basic reproductive ratio (R_0)

Fundamental to describing the epidemiology of the disease in many situations, especially for microparasites, is the concept of the basic reproductive ratio, R_0.

R_0 may be defined as the expected number of secondary cases produced in a completely susceptible population, by a typical infected individual during its entire period of infectiousness (Diekmann *et al*., 1990). A disease can invade and maintain itself in a host population only if $R_0 > 1$. If $R_0 < 1$ the disease cannot maintain itself and, under most circumstances, will become extinct. This is the threshold theorem of Kermack and McKendrick (1927), and it provides a convenient selection goal when breeding animals for resistance to a micoparasitic disease; the goal may be to reduce R_0 below 1. In the SIR model described above, R_0 is simply estimated as $\beta N/\gamma$.

For macroparasitic infections, the definition of R_0 is less straightforward. The number of parasites constituting the infection is important and R_0 must be formulated in terms of the dynamics of the parasitic population. For macroparasites, a definition of R_0 may be 'the average number of offspring (female offspring in a dioecious species) produced throughout the reproductive lifespan of a mature parasite that themselves survive to reproductive maturity in the absence of density-dependent constraints on population growth' (Anderson and May, 1992). This is directly equivalent to Fisher's definition of net reproductive rate for free-living species (Fisher, 1930). A formal definition of a parameter with threshold properties analogous to R_0, for macroparasitic infections, is given by Heesterbeek and Roberts (1994), who define a term called the basic reproductive quotient, Q_0. Q_0 has the same properties as R_0 in a microparasitic infection, i.e. $Q_0 > 1$ implies that the parasite can invade the population, and $Q_0 < 1$ implies that the parasite population is not expected to persist.

Genetic-epidemiological Models

Many epidemiological models have now been published for a wide variety of infectious diseases in farmed livestock, and it is beyond the scope of this review to even attempt to cover the range of models. Instead, so-called genetic-epidemiological models will be discussed, i.e. epidemic models that explicitly include host genetics.

Genetic-epidemiological models for microparasitic infections

The model of Stringer *et al*. (1998) was the first livestock microparasitic epidemic model to explicitly incorporate host genotype for resistance. In this model, specific alleles with Mendelian inheritance were defined to denote relative resistance or susceptibility to scrapie, reflecting PrP genotype associations often seen with scrapie resistance/susceptibility in sheep. The model predicted epidemics of several decades' duration which, with no intervention, would die out through natural selection of resistant genotypes. Importantly, the epidemic ended before the resistance allele reached fixation. Woolhouse *et al*. (1998) then used this model to compare the dynamics of breeding for scrapie resistance with other control strategies. These concepts were then extended from within-flock to between-flock models in order to predict the industry-wide impacts of

selection on PrP genotype to breed for scrapie resistance (Kao *et al*., 2001; Arnold *et al*., 2002; Gubbins and Roden, 2006). The value of this approach is that it helps to define end points for breeding programmes aiming to control classical scrapie, i.e. what proportion of the population is required to be of the resistant (ARR/ARR) genotype to protect the population as a whole, and the timescales over which this can be achieved (see Chapter 4, this volume).

A more generic approach to developing within-herd genetic-epidemiological models for livestock microparasitic infections was first illustrated by Mackenzie and Bishop (1999) and then developed in a stochastic framework by the same authors (MacKenzie and Bishop, 2001a, b), who parameterized their model for a real disease (porcine transmissible gastroenteritis) and included complex farm structures and population demographics. This work is based on the premise that while R_0 is often thought of as a property of the disease, it is equally well a function of the mean host genotype of an infected population. Therefore, selection to increase resistance may be thought of as selection to reduce R_0, which affects both the likelihood and severity of epidemics. The expected severity of an epidemic may be described by two parameters: the probability of an animal being infected during the course of the epidemic ($I = 1 - exp(-R_0 I)$) and the maximum proportion of animals infected at any one time ($Y_{max} = 1 - (1 + lnR_0)/R_0$). These parameters are shown plotted against R_0 in Fig. 3.1; for $R_0 > 5$, essentially all animals will become infected unless there is intervention. Additionally, the probability of an epidemic arising in a population is also a function of R_0. If there is a single initial infected animal, then the probability of no epidemic (i.e. the infected animal dies or recovers before infecting anyone else) is $1/(1 + R_0)$; the probability of a major epidemic (one that runs its expected course) is zero for $R_0 \leq 1$, otherwise $(R_0 - 1)/(1 + R_0)$; and the probability of a minor epidemic (one that dies out through stochastic events) is $R_0/(1 + R_0)$ for $R_0 \leq 1$, otherwise $1/(1 + R_0)$. These probabilities apply equally to homogeneous and genetically variable populations assuming an SIR model (Bishop and MacKenzie, 2003), and are shown plotted in Fig. 3.2.

Thus, in principle, selection for increased resistance has the double benefit of reducing the probability of an epidemic and reducing the severity of an

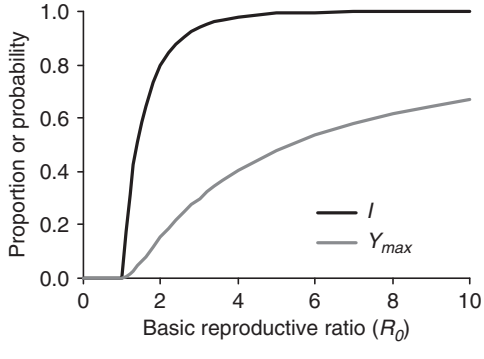

Fig. 3.1. The probability that an animal is infected during an epidemic (I) and the maximum proportion of animals infected at any time point (Y_{max}) as a function of the basic reproductive ratio.

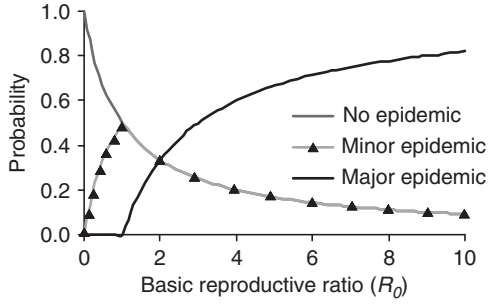

Fig. 3.2. Probabilities of no epidemic, a minor epidemic or a major epidemic, as a function of the basic reproductive ratio, given a single initial infected animal.

epidemic, should it arise. However, whether or not this is realized depends on the initial R_0 value and the means by which selection is achieved. For example, if R_0 is in the region of 1–4, selection should be effective in reducing risks and severities of epidemics. Conversely, for larger values (e.g. $R_0 > 5$), selection is unlikely to be feasible as even after many years of successful selection R_0 would remain sufficiently high so that an epidemic would still sweep quickly through the population (MacKenzie and Bishop, 2001b). However, major genes for resistance will make selection feasible, as seen in examples such as scrapie and various forms of *Escherichia coli* in pigs. Assuming a gene for complete resistance, the proportion of animals that must be resistant to protect the population as a whole, i.e. reduce R_0 below 1, is $1 - 1/R_0$ (MacKenzie and Bishop, 1999). This result is shown in Fig. 3.3, with generalizations presented by Bishop and MacKenzie (2003). The case of a single gene with two alleles is conceptually equivalent to the proportion of animals requiring vaccination, under the assumption of a completely effective vaccine that is used in a fully mixed population (Anderson and May, 1992). The key result for animal breeders is that epidemic risks become negligible even when genetically susceptible animals remain in the population, as seen in the scrapie modelling studies. This result is of importance when determining breeding strategies incorporating a disease resistance gene, as it implies that it is not necessary to take the beneficial allele to fixation. This differs from the production trait situation where it is usually desirable to increase the frequency of a desirable allele as far as is feasible.

The modelling results described so far have been derived for standard SIR models. These results extend in several ways, for example to more complex models and to models parameterized for specific diseases. For example, to determine the traits that might be most beneficial to select to achieve enhanced disease resistance, Nath *et al*. (2004) explored the impact of varying the transmission coefficient (i.e. resistance to infection), latent period, recovery period, mortality rate and the period of loss of immunity on overall epidemic outcomes. The critical parameters influencing the transmission of infection, and hence disease incidence, were the transmission coefficient, the latent period and the recovery period, whereas the period of loss of immunity had only trivial effects. Given that quantitative trait loci (QTL) or genetic markers are trait-specific,

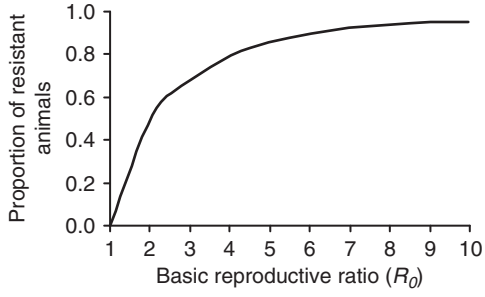

Fig. 3.3. The proportion of genetically resistant animals required to protect the population as a whole, as a function of the basic reproductive ratio.

these results may help researchers to focus on traits and QTL to target for maximum impacts on resistance. For example, susceptibility to infection might reasonably be considered a function of the innate immune response, whereas recovery rate is more closely related to acquired immunity. Apart from the scrapie models described above, notable disease-specific models that include consideration of host genotype include bovine mastitis (Detilleux, 2005) and ovine footrot (Nieuwhof *et al*., 2009). In both cases, although some complexity is added by formulating models that mimic specific diseases, it is clearly shown how host 'genetic management' can impact upon the disease epidemiology and therefore assist in disease control.

Finally genetic-epidemiological models may be used to explore impacts of genetic heterogeneity on disease risks, as outlined by Springbett *et al*. (2003). Comparing populations with one, two and many genotypes, it was demonstrated that genetic heterogeneity had no impact upon R_0, but it affected the variability of this parameter. Consequently, increased genetic heterogeneity was associated with an increased probability of minor epidemics and decreased probabilities of both major (catastrophic) epidemics and no epidemics. Additionally, heterogeneity per se was associated with a breakdown in the expected relationship between R_0 and epidemic severity, which is derived assuming homogeneous populations, with increasing heterogeneity generally resulting in slightly fewer infected animals than expected. Thus, the modelling results suggest that while heterogeneous populations are not expected to suffer fewer epidemics, they are less likely to suffer catastrophic epidemics.

Genetic-epidemiological models for macroparasitic infections

Macroparasitic infections are generally more complex than microparasitic infections, particularly with the need to model between-animal variation in infection severity. Hence the models are generally disease specific, and in the livestock context they are almost exclusively limited to ruminant nematode infections. One of the first authors to acknowledge the influence of host genotype for resistance and attempt to quantify the effects of host genotype on disease epidemiology was Barger (1989). Using the model for nematode

infection in sheep subsequently published by Barnes and Dobson (1990), he demonstrated that the assumed genetic resistance of the sheep population, as assessed by faecal egg count (FEC), should indeed regulate the disease epidemiology. In simple terms, sheep that are more resistant lead to lower pasture larval contamination and these sheep require fewer anthelmintic treatments to keep parasite burdens below a pre-defined threshold. Therefore, a positive feedback loop is created: resistant sheep put fewer parasites back on to the pasture, which then lowers the subsequent challenge to the sheep, resulting in even lower parasite contamination on the pasture and so on. That this is a true epidemiological change in the disease dynamics is readily seen from the fact that all sheep subsequently grazing the pasture will benefit from the reduced larval challenge, not only selected 'resistant' sheep but also unselected 'susceptible' sheep.

These concepts were developed further by Bishop and Stear (1997), who modelled the epidemiological consequences of selecting for reduced FEC in sheep. Their model defined heritable between-animal variation for pasture intake (hence larval intake) and for each major interaction between the parasite and the host. This created heritable variation in FEC, which mimicked field data and allowed the effects of selection to be investigated. Selection responses predicted by this model were always greater than those predicted by quantitative genetic theory. Although the actual responses depended on the grazing management assumptions, they were up to two times greater than predicted. The reduced pasture contamination is also predicted to impact on performance, as shown by Bishop and Stear (1999), who developed their model to include growth rate of the host sheep and growth rate losses due to infection. Figure 3.4 shows responses in live weight gain from selection of reduced FEC for two situations: (i) where the sheep graze the same pasture each year, and hence fully exploit the epidemiological effects; and (ii) where they graze different but equally contaminated fields. Fully exploiting the epidemiological effects can lead to large productivity benefits; in this case the correlated improvements in productivity were sometimes greater than the direct responses from selection for productivity. Crucially, these correlated epidemiological benefits are not symmetric as selection for increased productivity will not lead to correspondingly large improvements in resistance.

Experimental verification of these modelling results is difficult as it requires resistant and susceptible animals to be grazed separately under equivalent environmental conditions. However, the experimental results of Gruner *et al*. (2002) and Leathwick *et al*. (2002) both suggest that epidemiological benefits do arise from decreased pasture contamination, and these indeed are in addition to the direct effects of selection. Together, the modelling and experimental results suggested can be used to derive the correct weighting to be placed on disease resistance and productivity traits in breeding programmes.

Genetic-epidemiological models of nematode infections in sheep have recently been revisited, with a greater focus on nutritional and immunological factors affecting host responses (Vagenas *et al*., 2007c) and an exploration of the assumptions underlying the models (Doeschl-Wilson *et al*., 2008). The purpose of this exercise is to broaden the scope of the questions that the

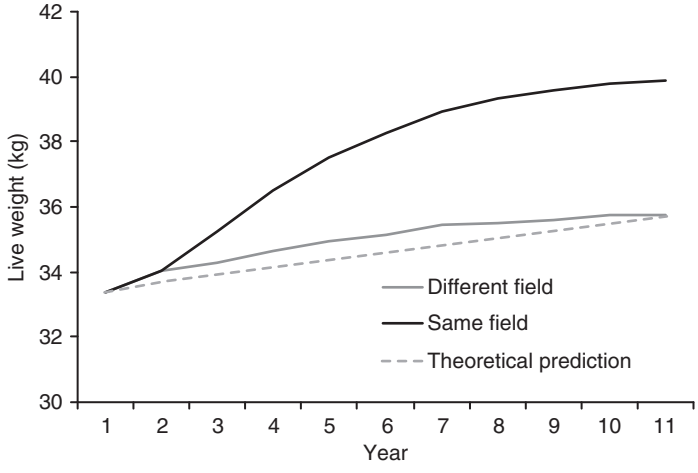

Fig. 3.4. Predicted responses in live weight resulting from selection for increased resistance to nematode infections in sheep, as assessed by faecal egg count. The 'same field' scenario assumes that epidemiological effects are fully exploited. 'Different field' assumes that different but equally contaminated pastures are grazed each year. The 'theoretical prediction' is the prediction obtained from quantitative theory, ignoring epidemiological effects. The difference between the theoretical prediction and the other lines represents the epidemiological contribution to the apparent selection responses.

models can address, potentially using them to devise integrated parasite control strategies that include many aspects of control, including host genetics, nutrition, anthelmintic intervention and grazing management. The complexity of such strategies necessitates the use of models and, in return, the development of these models quickly identifies areas where knowledge is lacking and therefore potential areas of future research.

Epidemiological Models: Incorporating Parasite Genetics

Much of the initial effort in disease modelling was put into modelling the parasite genotype rather than into the host genotype. As mentioned earlier, one of the major uses of disease epidemiology models has been to investigate the evolution of drug resistance in the parasite, especially for anthelmintic resistance in nematodes (e.g. Dobson *et al*., 1987, 1996; Gettinby *et al*., 1989; Barnes and Dobson, 1990; Echevarria *et al*., 1993; Leathwick *et al*., 1995). These models generally assume that resistance of the nematode to the drug is controlled by a single biallelic locus. The mode of inheritance (i.e. recessive or dominant) is known to differ for different families of anthelmintics, and it may even be dose-dependent (Dobson *et al*., 1996). Differential fitness for the different parasite genotypes can also be incorporated (Leathwick *et al*., 1995). These models are very useful for devising strategies to extend the life of available anthelmintics. Importantly, they demonstrate that dosing frequency per se is a poor indicator

of selection pressure for resistance (Leathwick *et al*., 1995), and that comprehensive modelling approaches are required to devise dosing strategies to minimize the evolution of anthelmintic resistance. Such models have been published recently by Cornell *et al*. (2003) and Gaba *et al*. (2006), combining not only parasite genetics but concepts such as spatial aggregation of eggs or larvae on pasture and parasite *refugia*. Parasites in *refugia* are those that are not subjected to anthelmintic selection pressure, e.g. those on pasture or in non-treated hosts, and Leathwick *et al*. (2008) give an example in which model predictions of parasite population dynamics, as affected by using untreated adult animals as a form of *refugia*, are partially verified by experimental results.

A major longer-term concern with the selection of domestic animal populations for resistance to specific diseases is the co-evolution of the pathogen in response to the genetic changes in the host. This issue has been investigated in detail in plants but to a lesser extent in animals. Traditionally, a simple gene-for-gene model has been used, where it is hypothesized that for each gene determining resistance in the host there is a corresponding gene for virulence in the parasite (Schmid-Hempel and Koella, 1994). This is a complex theoretical area, with Anderson and May (1992, p. 653) concluding that 'both theory and empirical evidence indicate that the coevolution between parasites and hosts can follow many paths, depending on the relationships between virulence and transmissibility of the parasite, and the cost to the host of evolving resistance'.

Although microparasitic parasitic coevolution models of relevance to livestock appear to be lacking, analogies can be drawn from the work of Gandon *et al*. (2001), who used an epidemic model to predict parasite evolutionary potential against different types of vaccines, especially 'imperfect' vaccines that have less than 100% efficacy. The model was applied to anti-malarial vaccines; however, the arguments would be equally valid for livestock diseases, such as Marek's disease in chickens, where each new generation of vaccine has apparently resulted in a new, more virulent (pathogenic) strain of the virus (Witter, 1998) (see Chapter 5, this volume).

The model of Gandon *et al*. (2001) was a super-infection epidemic model, accounting for the dynamics of both the original strain of virus and a mutant variety. Vaccines with 80% efficacy but different modes of action were considered: (i) anti-infection, analogous to host genetic susceptibility; (ii) anti-population growth; (iii) anti-transmission, analogous to host genetic infectiousness; (iv) anti-toxin, analogous to host genetic tolerance; or (v) a combination of these modes of action. The vaccine mode of action had marked impacts on predicted rates of pathogen evolution, with the modes of action analogous to resistance and infectiousness having the least deleterious impact on pathogen virulence. However, under some circumstances, an imperfect vaccine was predicted to have deleterious long-term impacts on disease-related deaths, due to the selection pressure it placed on parasite evolution.

The methodology of Gandon *et al*. (2001) provides a potential starting point for considering parasite evolution in an animal breeding context, a topic that has yet to be satisfactorily addressed. However, it should be recognized that when considering parasite evolution, absolute risks are very difficult to predict; it is the relative risks, compared with other disease control strategies, that are more tractable.

Some of the factors affecting potential rates of parasite evolution have been considered by Bishop and MacKenzie (2003). A primary factor will be the mechanism of resistance (or tolerance), analogous to the mode of action of the vaccine modelled by Gandon *et al*. (2001). It is notable that for a number of tropical diseases where the host and parasite have coexisted over long time periods, it is host tolerance rather than resistance that is observed. More generally, one may expect large differences in parasite co-evolution rates between genetic selection of the host and control procedures such as chemical intervention that kill the majority of the parasite population. The former process is gradual, with weak selection pressure being placed on the parasite, whereas the latter places intense selection pressure on the parasite population. Moreover, one may expect large differences between macro- and microparasites, merely as a consequence of the difference in generation intervals. For many macroparasites, the generation interval of the parasite is often an appreciable proportion of the generation interval of the host. A further factor is the genetic heterogeneity of host resistance. Resistance due to a single gene will arguably pose less of a challenge to the parasite than the multifactorial resistance, such as is seen for resistance to nematodes. This will tend to make major genes or QTL for resistance potentially a more risky means of controlling disease than polygenic resistance. However, the risks of parasite evolution must be placed in context with those incurred with alternative control measures.

Conclusions

While there has been considerable effort in modelling diseases in domestic animal populations, the use of these techniques for investigating host genetic effects and selection for disease resistance is still in its infancy. Moreover, the realization among animal breeders that epidemiological effects may contribute to apparent selection responses is only just beginning. Epidemiological models will become especially valuable when they can be used as a tool for animal breeders as well as a tool for understanding the disease itself. Two obvious uses for such models in a breeding context are: (i) deciding upon the feasibility of selection in the first instance; and (ii) using the models to help to derive relative economic weights for production and disease resistance traits. Situations are easily envisaged where the importance placed on disease resistance is a function of the prevalence of the disease in that environment. Non-disease-specific models are valuable for gaining general insight into the disease processes, as seen in the microparasitic models described above; however, models that are specific to the disease of interest will always be required.

Perhaps the major current requirement is the development of fully integrated models that include both host and parasite genetics. Parasite evolution in response to both chemical treatment and change in the host genotype needs to be considered. In the longer term, with greater emphasis on biological and vaccine control, models need to be developed that describe evolution in response to these control methods as well. Developing these fully integrated models will help in the devising of sustainable control strategies that combine several approaches for disease control.

In summary, it can be seen that developing disease models that describe the host genotype for resistance is an exciting new area of research. It will have practical benefits to the animal breeder and it will also provide additional insight into the dynamics of disease problems in domestic animal populations.

Acknowledgements

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II Viruses and TSEs

4 Transmissible Spongiform Encephalopathies

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Summary

This chapter briefly summarizes the impact of transmissible spongiform encephalopathies (TSEs) in sheep, goats, cattle and deer, and draws together the evidence for host genetic variation in resistance to such diseases. TSEs are fatal degenerative diseases which affect the central nervous system of many mammals and are characterized by long, asymptomatic incubation periods. Despite the fact that the agents causing TSEs are not fully understood, host genetic variation in TSE resistance is well characterized, particularly in sheep and goats affected with classical scrapie. In these species, various polymorphic forms of the *PrP* gene are strongly associated with disease incidence. For example, in sheep there are well-documented associations between PrP genotype and scrapie susceptibility or incubation period, and these apply to the majority of scrapie outbreaks and the majority of breeds. The standard resistance model is based on amino acids with significant worldwide frequencies at codons 136, 154 and 171, with the commonly seen haplotypes being ARQ, VRQ, AHQ, ARR and ARH. In turn, these combine to form 15 genotypes, with ARR/ARR being the most resistant to classical scrapie and VRQ/VRQ the most susceptible. In many countries breeding schemes based on PrP genotype have been successfully implemented to reduce the incidence of scrapie in sheep. Additionally, there has been careful monitoring of the genotypes of recent scrapie cases in order to reveal any developing problems such as the emergence of new forms of scrapie. Potential issues that may arise with PrP-based selection to reduce the incidence of scrapie in sheep and goats are discussed in this chapter.

Introduction

Transmissible spongiform encephalopathies (TSEs) are degenerative diseases which affect the central nervous system of many mammals. They include scrapie in sheep and goats, bovine spongiform encephalopathy (BSE) in cattle and Creutzfeldt–Jakob disease (CJD) in humans. TSEs are fatal and are characterized by long, asymptomatic incubation periods, which, in ruminants and humans, may last for years. Scrapie has been endemic in sheep in Europe for

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at least 250 years and, like other TSEs, can be transmitted from affected to healthy animals by experimental injection or feeding of diseased tissues. BSE, on the other hand, was first recorded in the 1980s, but much of the early understanding of this cattle disease came from the background of research on sheep scrapie in its laboratory-mouse-adapted forms.

Diagnosis of TSE is based on clinical signs and post-mortem brain pathology. Common features in fixed brain sections include the widespread formation of membrane-bound vesicles or vacuoles (the 'sponge' in spongiform) in cell bodies of nerve cells, vacuolation of the extracellular space and a proliferation of astroglial cells in the absence of demyelination or other overt inflammatory responses. These features are most pronounced in the terminal stages of disease and it is very difficult to diagnose TSE by histopathology in mammals that are not exhibiting symptoms.

More reliable as a preclinical diagnostic test is the presence, in tissue sections taken from affected individuals, of aggregated abnormal forms of a host protein called prion protein (PrP). During the preclinical phase, the normal protein, PrP^{C}, is, by an unknown mechanism, changed in conformation to become the aggregated, partially protease-resistant form, PrP^{SC}. This disease-specific marker has been found in peripheral tissues suitable for biopsy over a year before scrapie signs have developed in sheep.

The agent responsible for causing TSEs is unusual. This pathogen has some of the properties of a conventional virus but can survive normal virucidal procedures such as prolonged exposure to formalin, dry heat and some regimes of autoclaving. Biochemical studies have indicated that the PrP^{SC} protein is usually associated with infectivity and this has given rise to the prion hypothesis which suggests that this protein may be the sole constituent of the infectious particle. There is considerable evidence to support the idea of an infectious protein aetiology. However, while still recognizing the importance of PrP in these diseases, some researchers in the field propose that there are additional factors involved. The molecular structure of the pathogen is therefore controversial and it can only be convincingly detected by transmission to other animals.

Breeding for disease resistance is the most powerful means of controlling scrapie in sheep. The *PrP* gene in many polymorphic variants has been shown to be genetically linked to disease incidence both in experimental TSE in sheep and goats and in natural scrapie in a wide range of sheep breeds. This chapter reviews the current knowledge of the genetics and biochemistry of TSE susceptibility in sheep and goats and describes the reasons why such advances have not proved to be so useful with cattle and BSE.

The TSE Agent

The agents causing TSEs are remarkable pathogens. Scrapie has some of the biological properties of a conventional virus, for example heritable strain characteristics and host range limitations, but scrapie infectivity can survive normal virucidal procedures such as exposure to formalin, dry heat and autoclaving.

Biochemical and physical studies of scrapie infectivity have shown that protein is an essential part of the infectious particle and many believe that a protein is the sole factor necessary to cause disease. The abnormal form (PrP^{SC}) of the host protein (PrP^{C}) has been found to be closely associated with infectivity and it is PrP^{SC} that is regarded by proponents of the prion hypothesis as forming part, or all, of the infectious entity, either as an infectious protein (Prusiner, 1982) or simply as a genetic disease (for review see Prusiner and Scott, 1997). Although PrP^{Sc} is used as a marker for infection, it can be undetectable in certain circumstances where animal bioassay has shown infectivity to be present (Barron *et al*., 2007). Others have proposed that PrP^{SC} protects the pathogen and that the heritable scrapie strain-specific information is carried on another molecule, probably a nucleic acid (Bruce and Dickinson, 1987). This is the virino hypothesis, and there are still those who suggest that there is evidence for the involvement of a conventional virus (Manuelidis *et al*., 1987; Diringer, 1995; Dron and Manuelidis 1996). At the very least, PrP^{SC} is a disease-related marker and is useful in post-mortem diagnosis and in the development of preclinical biopsy procedures (Schreuder *et al*., 1996). The presence of infection is only properly demonstrable by transmission of disease to other animals, usually laboratory rodents. Surveillance and control of TSE infection is therefore problematic.

Clinical Signs and Pathology

The best studied of the TSEs is scrapie that occurs naturally in sheep and goats. Written descriptions of scrapie (and complaints about lack of government intervention to control the disease) have been documented for over 250 years in various parts of Europe, including England and Germany (Parry, 1984). Scrapie is a notifiable disease in European Union countries but the exact number of cases occurring each year is not known for certain, and there is undoubtedly under-reporting. The intensive surveillance schemes recently adopted to detect signs of BSE infection in sheep have resulted in the discovery of a previously unknown form of scrapie, now called atypical scrapie and epitomized by the index case Nor 98 (Benestad *et al*., 2003). To avoid confusion, the original type of scrapie is sometimes known as classical scrapie. Several countries are regarded as being free of classical scrapie disease, notably Australia and New Zealand, which have stringent procedures controlling the import of new sheep bloodlines, including many years of quarantine, in order to keep scrapie out. However, atypical scrapie seems to have a more widespread occurrence and it is unclear whether it is naturally transmissible (Simmons *et al*., 2009).

Clinical signs of scrapie in sheep (Dickinson, 1976) can last from two weeks to 6 months and begin with mildly impaired social behaviour such as unusual restlessness and signs of nervousness. In later stages of the clinical course, the general condition of the animal begins to deteriorate, sometimes accompanied by a change in fleece colour. Pruritis (itching with resultant inflammation) can result from the animal scratching its body against fence posts or

biting the affected area (Parry,1984). Typically this occurs around the base of the tail and, occasionally, the whole of the side of the body. In the final stages of scrapie, although the appetite may appear normal, the animals lose the ability to feed themselves and their condition really starts to degenerate. Scrapie does not seem to alter reproductive capacity until muscle wasting interferes with the ability to move. Lambs can be born successfully to mothers in the clinical phase of the disease and rams remain fertile and active even when showing clinical signs of scrapie (Parry, 1984; Foster & McKenzie, unpublished).

Reported clinical descriptions often vary, however. In a group of scrapie-affected sheep from Shetland between 1985 and 1991 (Clark and Moar, 1992), most animals showed signs of pruritis and emaciation; others had pruritis, emaciation *and* hyperaesthesia (oversensitivity); and yet others showed all these signs plus ataxia. In a report of clinical signs in some Japanese sheep (Onodera and Hayashi, 1994), some animals (Suffolks and Corriedales) showed signs of pruritis but others (Corriedales) had died for no obvious reason and scrapie was diagnosed after histopathological examination. These differences both in symptoms and clinical phase may simply be due to breed characteristics or may indicate the presence in the field of different strains of scrapie.

It is very difficult to detect, by histopathology, animals with scrapie that are not visibly affected by the disease. At terminal stages, however, common neurological lesions in the brain include neuronal degeneration with the formation of vacuoles, proliferation of astroglial cells but no demyelination or other overt inflammatory responses. Vacuolation is not present in the same parts of the brain in all scrapie cases; for example, one study of scrapie-affected sheep in Britain described seven different patterns of vacuolation (Wood *et al*., 1997) across ten brain regions. However, a more detailed study showed that vacuolation was subject to great variation depending on breed, PrP genotype and, possibly, scrapie strain. The considerable individual variability meant that vacuolation cannot be used as a strain-typing method in sheep (Begara-McGorum *et al*., 2002).

A much more reliable method of diagnosis is the detection of the PrP^{SC} protein, either by immunohistochemistry of brain or lymphoid tissues, or by Western blot analysis of tissue extracts. Patterns of PrP^{SC} deposition (also called PrP^{D}) can be used as a method of TSE strain typing in sheep (Gonzalez *et al*., 2002, 2003). Indeed, atypical scrapie (Nor98) was first detected by its novel pathology in brain, including PrP^{SC} accumulation in the cerebellum, unlike classical scrapie where this is seldom the case (Benestad *et al*., 2003; Bruce *et al*., 2007). PrP^{SC} can also be detected in the preclinical phase in lymphoid tissues such as tonsil (Schreuder *et al*.,1996) and rectal mucosa (Gonzalez *et al*., 2006) and is therefore of greater potential interest as a diagnostic test than is vacuolation.

Cattle affected by BSE become very difficult to handle and show increasing signs of ataxia, altered behaviour with fear and/or aggression and sensitivity to noises and to touch. Affected animals spend less time ruminating than healthy cattle (Austin and Pollin, 1993), although their physiological drive to eat appears to remain normal. Several studies have noted that BSE cattle have low heart rates (brachycardia) that may either be related to the low food

intake associated with reduced rumination or indicate that there is some damage to the vagus during disease development (Austin *et al*., 1997). BSE-affected cattle also show significant neuronal loss in the brain (Jeffrey and Halliday, 1994), and the appearance of vacuolar lesions in brain sections is very similar to that seen in sheep scrapie. BSE was confirmed as a TSE by the demonstration of the diagnostic TSE-related PrP fibrils in brain extracts (Hope *et al*., 1988) and by transmission of the disease to mice (Bruce *et al*., 1994).

TSE Genetics and Breeding for Resistance

In this section the other feature of PrP will be described in some detail, namely the association of various polymorphic forms of the *PrP* gene with disease incidence and the ability to make use of this association in breeding for TSE resistance. Figure 4.1 shows a model of the PrP molecule and the position of important amino acid polymorphisms that have been associated with disease incidence.

Genetic control of TSEs in sheep

The *PrP* associations with scrapie resistance in sheep are most thoroughly investigated and understood for classical scrapie. Although the underlying mechanisms are unknown, there are specific rules that relate the *PrP* genotype to scrapie susceptibility and incubation period. In the majority of scrapie outbreaks and the majority of breeds, a standard model using a three-codon *PrP* genotype applies. The standard model is based only on amino acids with significant worldwide frequencies at those codons: codon 136 has two possible amino acids, A and V; codon 154 also has two, R and H; and codon 171 has three possible amino acids, Q, R and H. These polymorphisms combine to form five haplotypes (referred to as alleles for convenience) (ARQ, VRQ, AHQ, ARR, ARH), and in turn these alleles combine to form 15 genotypes (ARR/ARR, VRQ/ARQ, ARH/AHQ, etc.) (Dawson *et al*., 1998; Goldmann, 2008).

For the purpose of scrapie control, five risk groups (Types 1, 2, 3, 4 and 5) for classical scrapie are defined based on these 15 genotypes, and these

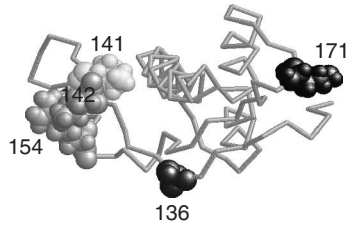

Fig. 4.1. Model of the C-terminal part of prion protein with highlighted amino acid positions that are polymorphic in sheep and goats and associated with TSE.

categories are applied in breeding and eradication programmes in the member states of the European Union and beyond. For example, the programme in the UK is known as the UK National Scrapie Plan. As shown in Table 4.1, the genotypes are placed in a series of groups which represent a refinement of the original risk groupings produced in 1998 (Dawson *et al*., 1998). The highest risk group (Type 5) contains four genotypes and represents animals that are at greatest risk of developing scrapie (Baylis *et al*., 2004). The genotype within Type 5 which is at greatest risk is VRQ/VRQ. Although scrapie was in the past often regarded as a genetic disease, as the risk in this genotype is so high, it has been shown that VRQ/VRQ sheep can survive into old age when their environment is free of scrapie infection (Hunter *et al*,. 1997; Foster *et al*., 2006). The other three genotypes in the Type 5 group are VRQ/ARQ, VRQ/ARH and VRQ/AHQ. The risk estimates for VRQ/ARQ and VRQ/ARH heterozygotes are also high, although for VRQ/AHQ sheep the risk of scrapie is surprisingly low when estimated from UK data, suggesting that the AHQ allele confers partial resistance (Baylis *et al*., 2004). The Type 4 risk group comprises animals of the VRQ/ARR genotype that are at a lower risk of being affected by scrapie than Type 5 animals. Because their genotype comprises the most resistant and the most susceptible allele, they are capable of producing offspring in the two extreme groups. Type 3 animals have average resistance to scrapie and the same is true for their offspring, which will always belong to the Type 3 group if matings are restricted to other members of the same group. The six genotypes in this group include the wildtype (wt) genotype ARQ/ARQ. The susceptibility of ARQ/ARQ animals varies between different scrapie outbreaks: for example, while ARQ/ARQ homozygotes in the UK have

Table 4.1. Sheep PrP genotypes and risk of classical natural scrapie

Group/Genotype	Risk of scrapie[a]
1. ARR/ARR	Most resistant to scrapie
2. ARR/ARQ ARR/ARH ARR/AHQ	Resistant to scrapie but offspring may be susceptible depending on genotype of other parent
3. AHQ/AHQ ARH/ARH ARQ/ARH AHQ/ARH ARQ/AHQ ARQ/ARQ	Average risk of scrapie in these sheep and in their offspring
4. ARR/VRQ	Susceptible to scrapie but could be used as a breeding source of the ARR allele associated with resistance
5. ARQ/VRQ ARH/VRQ AHQ/VRQ VRQ/VRQ	Highest susceptibility to scrapie in these sheep and their offspring

[a]Information adapted from the Defra National Scrapie Plan for Great Britain, Ram genotyping scheme (http://www.defra.gov.uk).

only average susceptibility, this genotype has been reported to have similar risk to VRQ carriers in other European countries (O'Doherty *et al*., 2002; Billinis *et al*., 2004; Luhken *et al*., 2007). In the Type 2 group, animals of genotypes ARR/ARQ, ARR/AHQ and ARR/ARH are quite resistant to scrapie but their offspring can be of a higher (Type 3) risk classification. Occasionally Type 2 animals are found with scrapie whereas Type 1 animals (ARR/ARR) are resistant to classical clinical scrapie disease, although not to experimental exposure to BSE (Houston *et al*., 2003).

Given the *PrP* association with classical scrapie, it becomes important to know the allele frequencies of the major variants in different populations, and considerable effort has gone into genotyping large numbers of sheep. The average allele frequencies for all countries where data are published are 56% ARQ, 30% ARR, 6% AHQ, 5% VRQ and 3% ARH (for references see Goldmann, 2008). There are large regional and breed variations, however. For example, there is a lower than average frequency (~22%) of the ARR allele in the Mediterranean countries. These figures are likely to change over following decades when genotyping and breeding programmes currently under way take effect. Almost 25% of all ARQ alleles have additional polymorphisms, and TSE genetics research is only at the beginning of the process of determining their disease association, risk classification and utilization in breeding programmes.

There are some important differences between classical and atypical scrapie in terms of host genetic susceptibility. Most cases of atypical scrapie are found in animals of PrP genotype risk groups 1–3 (Table 4.1), which are the groups that show low susceptibility to classical scrapie. Atypical scrapie susceptibility is associated with *PrP* codons 141 (L or F) and 154 (R or H). In genetic studies, sheep of AHQ/AHQ and AHQ/ARQ genotypes were over-represented in disease groups compared with healthy controls. Further study revealed that an additional codon (141) was apparently affecting susceptibility to atypical scrapie. The F141 allele, which is in strong linkage disequilibrium with ARQ, conferred higher susceptibility to atypical scrapie than the wt L141 allele. Surprisingly, ARR/ARR animals, which are highly resistant to classical scrapie, are susceptible to atypical scrapie. Allele frequency analysis suggests that sheep with genotypes conferring susceptibility to atypical scrapie exist in most sheep populations and genetic screening might be used for assisting in the control of atypical scrapie (Moum *et al*., 2005; Saunders *et al*., 2006; Arsac *et al*., 2007; Luhken *et al*., 2007).

Genetic control of TSEs in goats

The prevalence of natural scrapie in goats is much lower than in sheep. Whether this is due to different relationships between PrP genotype and resistance or different agent strains is currently under investigation. Codons 136 (A) and 171 (Q) are not polymorphic in goats, but codon 154 shows the same polymorphism (R or H) as in sheep, and codon 154 appears to have a similar effect in classical and atypical scrapie susceptibility in goats as it does in sheep. This implies that the mechanisms underlying the interactions between *PrP* and the

infectious agent could be the same in different species. The codon 142 isoleucine (I) to methionine (M) polymorphism was the first goat-specific polymorphism to be associated with the incubation period of experimental scrapie, and M142 appears to be associated with higher resistance (Goldmann *et al*., 1996; Barillet *et al*., 2009). Other caprine polymorphisms with association to lower scrapie risk are H143R, N146S/D and Q222K, but numbers are still too small to accurately quantify their impact on resistance to classical scrapie strains. Animals with genotypes HR at codon 143, NS and ND at codon 146 and QK at codon 222 all seem to be protected more compared with HH143, NN146 and QQ222 homozygote animals (Billinis *et al*., 2002; Papasavva-Stylianou *et al*., 2005; Acutis *et al*., 2006; Vaccari *et al*., 2006).

Genetic control of BSE in cattle

In cattle, variation in *PrP* differs from that seen in sheep or goats. The N-terminus of *PrP* normally contains five copies of a unique glycine-rich peptide sequence often simply referred to as repeats. The number of repeats is polymorphic in all domestic ruminants, with the wt alleles containing five repeats. In cattle *PrP*, alleles with four to seven repeats are known. No association between BSE susceptibility and cattle *PrP* alleles with repeat variation has been found (Hunter *et al*., 1994). As yet, no other *PrP* polymorphisms have been identified in cattle. However, recently discovered polymorphisms in the *PrP* promoter – the region of DNA that controls levels of expression of the gene – may be associated with susceptibility to BSE in some herds. A possible effect has been observed to be associated with a 12 base pair (bp) deletion allele (12del). Homozygous 12del/12del and heterozygous 12del/wt animals appear to be more at risk of developing BSE than homozygous wt/wt animals (Juling *et al*., 2006). This may ultimately result in a model linking gene expression, and therefore protein levels, with disease in tissues that are important for BSE infection.

Genetic control of TSEs in deer

Deer in the USA and Canada and in some restricted areas in South Korea are infected with another TSE called chronic wasting disease (CWD). Here, too, a *PrP* genetic component to susceptibility has been shown between CWD-affected and healthy white-tailed deer, in captive and free-ranging populations. The associated variations are, however, quite different from the ones described above for domestic ruminants. Polymorphisms Q95H and G96S are associated with reduced risk of CWD. Deer homozygote for SS in codon 96 as well as heterozygotes GS96 and GH96 are under-represented in CWD-affected populations (O'Rourke *et al*., 2004; Johnson *et al*., 2006). The incidence of CWD in mule deer has also been shown to be associated with a polymorphism but at codon 225 – S225F (Jewell *et al*., 2005). However, none of the deer PrP genotypes seems to confer full resistance to CWD. An association of polymorphism M132L

with CWD susceptibility in free-ranging and farmed CWD-affected wapiti (Rocky Mountain elk) is less strong, although this variation may lead to longer incubation periods (O'Rourke *et al*., 1999; Perucchini *et al*., 2008).

Potential and pitfalls

The advantages in using PrP genotyping in domestic sheep and goats to breed against TSE susceptibility is that within a number of years (depending on frequencies of genotypes) a flock/herd can be rendered free of clinical signs of TSEs. On the basis of present knowledge, it is believed that this approach will work in eliminating clinical classical scrapie in sheep and, as such, it is the most powerful measure of scrapie disease control available to the sheep breeder. Breeding for disease resistance in goats is becoming more likely in the near future as *PrP*–disease associations are gradually unravelled in current projects. As promising as this approach appears, it may reduce genetic diversity if applied without caution and it may not be applicable in rare breeds, where the genotype frequencies of resistance alleles could be too low for eradication programmes to succeed.

The potential disadvantages in changing PrP genotype frequencies in sheep by the use of ARR/ARR rams include, first, the possibility that there might, by chance, be undesirable changes in performance attributes. This problem is avoidable by means of selection on the basis of both PrP genetics and performance traits. Recent studies into the consequences of PrP genotype selection have been quite positive in that detrimental effects have not been documented for traits such as lamb survival, growth or carcass composition, ewe milk production, *Salmonella* resistance or wool traits in scrapie-resistant genotypes (Vitezica *et al*., 2007; Sweeney and Hanrahan, 2008; Gubbins *et al*., 2009). A second potential problem with selection on classical scrapie-resistant genotypes is that this could inadvertently result in selection for scrapie strains that could cause scrapie in ARR/ARR sheep. Atypical scrapie is the prime example of the potential problem. Although currently appearing at a low rate, it may, over time, become a more important strain than classical scrapie. Mixed-genotype flocks, avoiding only the most susceptible animals, may offer the best protection against the emergence of new natural scrapie strains.

An additional problem relates to the possibility that 'carrier' sheep may exist and, if so, they could pass on infection to other sheep. In this scenario, ARR/ARR sheep would not show clinical signs of scrapie but may harbour a silent or latent infection, posing a threat by maintaining the infectious agent and creating a potential situation in which a new strain, capable of causing clinical scrapie, could be selected. One of the earliest studies of scrapie in mice (Chandler, 1963) showed that incubation period was dose dependent. In other words, incubation period lengthened as the amount of infectivity in the inoculum was reduced. At very low levels of scrapie infection, replication may take such a long time to build up that disease does not develop within the animal's normal lifespan (Dickinson *et al*., 1975), Such a situation, if shown to exist in sheep (and as yet there is no proof of this), could lead to the maintenance of a low level of infection in a flock without any signs of clinical signs. In principle, such sheep may shed scrapie

infectivity for many years and be a source of infection for their flockmates. Cross-species persistence may also occur: hamster scrapie injected into mice does not produce disease but the hamster scrapie remains in the brain and spleen of the mice and can be recovered in a form still capable of infecting hamsters (Race and Chesebro, 1998). Very sensitive methods are being developed to reveal the presence of the infection associated with protein PrP^{SC} and these will, in the future, be used to study resistant sheep to give an indication of whether they are truly resistant or whether they harbour infection at subclinical levels.

In cattle, the option to control TSE disease by breeding for resistance is not yet available – there are no genetic markers associated with BSE resistance. BSE in the UK is in decline as a result of the physical measures taken to control cattle food along with the slaughter of any animal considered at risk of disease (Anderson *et al*., 1996).

The creation of animals without a functional *PrP* gene (often referred to as *PrP*-null or *PrP*-knock-out) has recently been considered, as *PrP*-null mice are resistant to the TSE agent. A *PrP*-null goat has been produced, demonstrating that this approach is possible, in principle, for domestic ruminants (Yu *et al*., 2009).

Genetic control of CWD in farmed mule and white-tailed deer could be possible but there is no easy application of genetics in free-ranging animals. This is a particular problem in North America where the disease epidemic is currently severe; however, this problem has yet to be encountered in European free-ranging deer.

Conclusions

In conclusion, the use of genotyping in breeding for TSE resistance is currently possible only in sheep. It should be encouraged, but not to the exclusion of other desirable traits. Large-scale testing of PrP genotypes has taken place throughout Europe and there has been careful monitoring of the genotypes of any scrapie cases which occur in order to reveal any developing problems such as the emergence of atypical scrapie. In addressing the problems and concerns of the sheep breeder, science continues also to address the remaining questions about the aetiology of TSEs – the unusual pathogens.

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5 Viral Diseases in Chickens

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Summary

Controlling infectious disease is one of the major issues facing poultry breeders, particularly since the introduction of high-density rearing. The poultry industry relies primarily on animal husbandry and vaccination for disease control. Commercial poultry vary in disease incidence for most of the important avian viral pathogens; thus, selection for enhanced genetic resistance to disease offers another added layer of control. Furthermore, chickens with enhanced disease resistance would help poultry-breeding companies gain market share, maintain consumer confidence and improve animal welfare. Due to the high costs associated with pathogen challenge and the biological complexity of disease resistance, selection has been limited to very few viral diseases where the primary control methods of animal husbandry and vaccination may fail. With the advent of the chicken genome sequence and related genomic technologies, there is enormous potential to identify specific genes, mutations and pathways controlling disease resistance. The ensuing knowledge can be utilized to produce commercial poultry resistant to many more diseases using genetic markers and other indirect methods of selection. This chapter highlights the technologies that are making this advance in biological knowledge possible, specific diseases where cutting-edge technologies are being applied and future prospects.

Introduction

Modern animal breeding programmes and production practices have been very successful in meeting the growing demands of consumers worldwide for high-quality, safe and affordable animal products. This trend is most evident for the poultry industry. For example, from 1957 to 2006, poultry production in the USA grew more than fivefold, making it the primary meat consumed in the USA and allowing the USA to become the world's largest producer and second largest exporter of poultry meat (United States Department of Agriculture (USDA), National Agricultural Statistics Service (NASS)). A similar

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trend was observed outside the USA where poultry meat production increased by 436% from 1970 to 2005, which compares favourably to the 186% and 57% increases for pork and beef, respectively (Food and Agriculture Organization (FAO) database).

Despite these great gains, several major issues confront the poultry industry today. New and reemerging infectious diseases are certainly at or near the top of the list. Avian influenza and exotic Newcastle's disease are just two viral pathogens well known to the public that harm the poultry industry through loss of birds, reduced public confidence and lost market accessibility via trade restrictions. Disease outbreaks or the potential for them to occur are enhanced by high-density chicken rearing and reduced genetic diversity from industry consolidation. Changes in animal husbandry and new vaccines have helped to alleviate some of the problems; however, improved or alternative control measures are still needed to address current diseases and impede emerging threats.

Prior to and certainly after the generation of the chicken genome sequence in 2004 (Hillier *et al*., 2004), the scientific emphasis on disease control has relied on cutting-edge technologies that provide fundamental information on the molecular basis for disease manifestation and control. From the genetics and breeding perspective, since the development of molecular genetic maps in the 1990s, the attention has focused primarily on the identification of specific genes or quantitative trait loci (QTL) that account for some or all of the resistance to a specific disease. Identification of these genes or genomic regions allows for linked markers that can be used to indirectly select for enhanced disease resistance, a process referred to as marker-assisted selection (MAS). MAS offers numerous benefits as it potentially allows for increased selection intensity, increased accuracy of selection, maintains or integrates new genetic variation and, most importantly, avoids the costs and issues associated with exposing elite flocks to a hazardous pathogen.

Given this emphasis, the main technologies being employed in molecular genetics are discussed; for other reviews of chicken genetics as it applies to disease resistance, see Schat and Davies (2000), Bumstead (2003), Cheng (2003), Soller *et al*. (2006) and Cogburn *et al*. (2007). Furthermore, specific diseases are reviewed to determine what has been garnered thus far using modern techniques. Finally, future developments that are likely to have a significant impact are briefly discussed.

Definition of Disease Resistance

To increase genetic resistance to disease, it is important to first define what is resistance to disease. As obligate intracellular pathogens, viruses must first enter the host cell since they require the host machinery to replicate. Thus, for viruses that can normally infect chickens, disease resistance in some cases means absolute resistance to infection at the cellular level and, consequently, the entire bird. This type of disease resistance is typically manifested when the

cellular receptor is altered such that it does not support viral attachment or entry. Consequently, resistance is against viral infection and can be considered as sterilizing or true disease resistance. Furthermore, in all such cases, genetic resistance is accounted for by a single gene; this can be tested *in vitro* to see whether the appropriate cell can be infected and, thus, does not require an intact immune system.

For the majority of viral diseases, disease resistance actually means tolerance to the effects of viral infection. As disease occurs when there is an unfavourable outcome of interactions between the host, pathogen and environments that favour pathogen growth and spread, disease-resistant birds would be ones that do not pass a certain threshold of the measured trait to be classified as diseased. Unlike resistance to viral infection, tolerance to disease is normally a complex trait involving many genes including those involved in the immune response. In addition, vaccines and other external forces (e.g. diet, stress) can influence the outcome of the interaction of the host and the pathogen.

Commonly-used Strategies to Identify Factors for Complex Disease Resistance

As previously stated, the primary method envisioned to implement or augment existing selection schemes, including enhanced genetic resistance to viral diseases, is MAS. Thus, genetic markers linked to genes or QTL that control viral disease resistance must be identified or located on the genome. The manner by which these genes or QTL are being tested or identified is heavily dependent on available information and technologies.

Testing of candidate genes is often a productive first approach to use when there is prior knowledge of the genes or pathways that confer resistance. In virtually all situations where disease resistance is complex, immune response genes are obvious choices. The major histocompatibility complex (MHC) is a region on chromosome 16 that contains three tightly linked regions known as *B-F* (class I), *B-G* (class IV) and *B-L* (class II), which control cell surface antigens, and many other key genes that influence the immune response (Kaufman *et al*., 1999). In contrast to mammals, the chicken MHC or *B* complex is simple with single dominantly expressed class I and class II genes. This may be one reason why the MHC is often associated with viral disease resistance in chickens, in contrast to mammals that express multiple class I and II genes (Kaufman and Wallny, 1996). Also, MHC haplotypes can often be quickly identified using either specific antisera or molecular markers, as there is minimal recombination within the complex (Fulton *et al*., 2006). Finally, given the importance of the MHC, chicken lines have been generated that differ only in the MHC haplotype (Abplanalp *et al*., 1985; Bacon *et al*., 2000). These *B* congenic lines greatly facilitate studies investigating whether or not there is an MHC influence on disease resistance. However, as there is only a single background strain, one has to be aware of possible interactions with non-MHC regions (Hartmann, 1989).

Other candidate genes that are frequently investigated with respect to viral disease are Toll-like receptors (TLRs). As part of the innate immune system,

TLRs recognize structures known as pathogen-associated molecular patterns (PAMPs) that are commonly found in microbes and viruses. A search of the chicken genome has identified two TLRs involved in viral recognition (Jenkins *et al*., 2007). Specifically, the chicken orthologs for TLR3 and TLR7 that recognize ssRNA and dsRNA, respectively, are present.

Testing of candidate genes is most powerful when the extent of linkage disequilibrium (LD) is minimal, as this reduces the chance that the gene being tested is acting as a linked marker, i.e. any observed association is more likely to be the result of the actual gene and not one that is linked. Because of this, candidate genes can be directly tested in commercial populations that have low levels of LD, due in part to being non-inbred and having relatively large effective population sizes.

For most quantitative traits including disease resistance, our level of understanding is limited; thus, comprehensive genome-wide QTL screens are required as the first step to gain more knowledge. The ability to screen most of the chicken genome emerged with the development of molecular genetic maps, especially those containing microsatellite markers, as microsatellites are highly informative and amenable to semi-automated genotyping (Bumstead and Palyga, 1992; Levin *et al*., 1994; Cheng *et al*., 1995; Groenen *et al*., 1998, 2000). However, the initial genetic maps were not very dense, which restricted resource populations being tested to those where the extent of LD was relatively large. This usually meant establishing pedigreed backcross (BC) or F_2 populations that were genotyped with markers spaced 20–40 cm apart; on the other hand, the wide spacing reduced the total number of markers needed. Then each marker and marker interval were tested to determine whether the inheritance of specific alleles influenced disease resistance in a defined population. To validate and fine-map QTL identified in the initial genome-wide screen, additional generations are required to reduce the extent of LD and test additional markers in the region.

The situation has changed rapidly with the identification of millions of single nucleotide polymorphisms (SNPs) (Wong *et al*., 2004), along with platforms that allow economical and high-throughput genotyping. As a result, resource populations that have very low levels of LD, such as commercial populations, are favoured. Rather than following the inheritance of specific alleles through families (i.e. identity by descent (IBD) mapping in some instances), association mapping is employed which analyses genetic markers by allele frequency or identity by state (IBS).

Another technological advance that has altered the questions that can be addressed, and the way in which they are addressed, is deoxyribonucleic acid (DNA) microarrays. First described in 1995 (Schena *et al*., 1995) and now widely applied, DNA microarrays contain thousands of probes placed at known locations which, when hybridized to type c ribonucleic acid (cRNA), give the relative expression of all the queried genes in a single step for that sample. When two or more samples are processed, one can identify genes that are differentially expressed and, in principle, infer the pathways that contain these genes. However, transcript profiling does not directly identify genetic effects

causing differences between individuals. To investigate genetic effects, the list of differentially expressed genes and the genes in the pathway can be considered as candidate genes, which can then be tested individually as described above or merged with QTL scans.

Genetic Resistance to Specific Diseases

Lymphoid leukosis

Avian leukosis viruses (ALVs) are a group of retroviruses that can induce tumours, the majority of which are lymphoid leukosis (LL) (Fadly and Nair, 2008). ALVs found in chickens are classified into six subgroups (A, B, C, D, E and J) based on virus-specific cellular receptors and virus envelope glycoproteins. All subgroups except E are exogenous ALVs. Genetic resistance at the cellular level to all exogenous ALVs, except ALV subgroup J (ALVJ), is known to exist.

Subgroup A (ALVA) is common and widespread in commercial chickens, and subgroup B (ALVB) is also somewhat prevalent. Prior to the late 1970s, selection of resistant birds or eradication of the virus from breeding flocks was difficult as the methods to detect virus infection were complex, expensive and not 100% accurate. The fact that birds did not succumb to ALV-induced tumours until 20 weeks of age or later, combined with vertical transmission of the virus from hen to chick, made control of ALVs even more difficult. Fortunately, in 1977, fast and economical methods were introduced that allowed the industry to eradicate ALVA and ALVB (Spencer *et al*., 1977), and this remains the primary control method.

Although genetic resistance is not being selected for, with the exception of ALVJ, it is known to exist both at the cellular level and for tumour progression. Given that sterilizing resistance to ALV subgroups A–E is simple and well defined, this would be the preferred method to implement. Molecular studies have identified the cellular receptor for all these subgroups as well as revealed alleles that confer genetic resistance, with the exception of ALVJ receptors. The tumour virus A (*TVA*) locus found on chromosome 28 encodes the cellular receptor for ALVA, which is a member of the low-density lipoprotein receptor (LDLR) family (Bates *et al*., 1998; Elleder *et al*., 2004a). Resistance to ALVA has been shown by two alleles. The first resistance allele, *TVA***R*, contains an SNP that results in a protein with very low binding affinity to the ALVA envelop. The other resistance allele, *TVA***R2*, has a 4-nucleotide insertion near the beginning of the coding sequence, which results in an altered protein. The *TVC* locus is also found on chromosome 28 and only ~1 cm from *TVA* (Elleder *et al*., 2004b). It encodes the ALVC receptor, which shows homology to butyrophilins, a member of the immunoglobulin superfamily (Elleder *et al*., 2005). The *TVC***R* allele contains a premature stop codon, which results in a non-functional receptor. Resistance to ALV subgroups B, D and E is controlled by the *TVB* locus (Adkins *et al*., 2000), which is located on chromosome 22 (Smith and Cheng, 1998) and encodes a protein that is related to the tumour necrosis factor receptor (TNFR) family.

There are several alleles that confer genetic resistance. *TVB***R* results from an SNP that generates a premature stop codon resulting in a non-functioning receptor and resistance to all three subgroups (Klucking *et al*., 2002). The *TVB***S3* allele confers resistance to subgroup E as the result of a different SNP.

As the receptors and the resistance alleles are known, genetic tests can be designed. Both medium- and high-throughput assays have been used to screen for genetic resistance to ALVB (Zhang *et al*., 2005). The results indicate that both broiler (meat-type) and layer (egg-type) commercial chicken lines can have resistant alleles, often at low frequency (Zhang *et al*., 2007). Selection for genetic resistance would provide an added level of protection, especially for layers as they are kept past the age where ALV-induced tumours normally become apparent.

Subgroup J represents a new and disturbing situation. ALVJ was identified in 1988 and became a significant problem in the 1990s (Payne *et al*., 1991). ALVJ is believed to be a recombinant virus between exogenous ALVs and ancient endogenous avian viruses (Benson *et al*., 1998; Silva *et al*., 2000). Like other new viruses, it rapidly spread and infected breeding flocks with rates as high as 87%. ALVJ induces myeloid leukosis, which is unlike the other ALV subgroups that primarily induce lymphoid leukosis. While the receptor for ALVJ has been identified and shown to be Na+/H+ exchanger 1 (NHE1), no cellular resistance has been observed although there have been field reports that suggest a genetic basis for differences in resistance to tumour formation (Chai and Bates, 2006). While resistance to ALVJ in chickens has not been identified, this unique opportunity should be explored given that the receptor gene is known, the continuing importance of the disease despite fairly successful eradication efforts, and the power and diminished costs of DNA sequencing.

Marek's disease

Marek's disease (MD) is a lymphoproliferative disease of poultry caused by the Marek's disease virus (MDV), an alphaherpesvirus (Osterrieder *et al*., 2006; Schat and Nair, 2008). The pathological manifestations of MD include immunosuppression, nerve enlargement and lymphomas that metastasize to various visceral organs. Due to the unpredictable and spontaneous vaccine breakdowns that result in devastating damage to poultry farms, MD remains one of the most serious chronic threats among infectious diseases to the poultry industry (Witter, 1997). Since the introduction of live vaccines against MD in the early 1970s, the condemnation rate attributed to MD has decreased dramatically (Witter, 2001). At present, practically all chickens raised in commercial farms are vaccinated against MD using live vaccines. These MD vaccines were revolutionary because they were among the first vaccines to prevent tumours, i.e. cancer vaccines. While these vaccines are very effective in preventing MD and tumour formation, they are not sterilizing. Thus, they do not prevent infection or shedding of pathogenic MDV (Witter, 2001).

Despite the success of vaccines in controlling MD, there is still great concern as new MDV strains with higher virulence have emerged, resulting in devastating losses to poultry farms. Moreover, even if birds do not succumb to MD, the immunosuppressive effects from MDV infection can exacerbate other diseases or allow infection by opportunistic pathogens. Based on pathogenicity shifts, it has been suggested that a new vaccine is useful for about 10 years (Kreager, 1996). Consequently, there is a need to augment current control methods. Genetic resistance in birds, which in this case is defined as failing to develop characteristic symptoms upon exposure to MDV, is one of the more exciting and promising avenues as not only does it provide additional control, but MD-resistant birds also respond better to MD vaccines.

Prior to modern genomics, the best understood mechanism for the involvement of genetic resistance to MD involved the MHC. Using antisera against the *B-G* (class IV) locus or using *B*-congenic chickens, it has been observed that the MHC is associated with MD resistance (Briles *et al*., 1977). In general, chickens with the *B**21 haplotype have been found to be more resistant than those with other *B* haplotypes (Bacon, 1987; Bacon and Witter, 1992). Other studies have allowed for the relative ranking of the other *B* haplotypes: moderate resistance, *B**2, *B**6, *B**14; susceptibility, *B**1, *B**3, *B**5, *B**13, *B**15, *B**19, *B**27 (Longenecker and Mosmann, 1981). *B* haplotype also influences vaccinal immunity as some haplotypes develop better protection with vaccines of one serotype than of a different serotype (Bacon and Witter, 1994a,b).

Apart from some candidate studies on genes (e.g. *RfPY* (Miller *et al*., 1996) and vitamin D receptors (Praslickova *et al*., 2008)), most genetic studies have utilized genome-wide QTL scans. Two studies utilized ADOL inbred lines 6 (MD resistant) and 7 (MD susceptible) that are both homozygous for *B**2, yet differ significantly in MD incidence. The resource population used by Vallejo *et al*. (1998) and Yonash *et al*. (1999) was unvaccinated F_2 progeny challenged with JM strain MDV. All chicks were measured for MD as well as a variety of MD-associated traits such as viral titre, number of tumours and length of survival. Using mostly microsatellite markers, 14 QTL (7 significant and 7 suggestive) were discovered, with additive gene substitution effects from 0.01 to 1.05 phenotypic standard deviations. Collectively, the QTL explained up to 75% of the genetic variance. Three of the QTL were associated almost exclusively with viraemia levels while the remaining QTL accounted for disease, survival, tumours, nerve enlargement and other disease-associated traits. This suggests that disease resistance occurs at least at two levels: initial viral replication and cellular transformation, which occurs later. It also highlights the added value of measuring several components as it may functionally separate a complex trait.

Using a (6 × 7) × 7 BC population, Bumstead (1998) mapped a single significant QTL on chromosome 1 that accounted for viral levels and tumour incidence; this QTL was not observed by Yonash *et al*. (1999). However, this region had conserved synteny with the mouse *CMV1* locus, which controls resistance to murine cytomegalovirus, another herpesvirus. Ly49H is the causative gene for *CMV1* resistance, and the encoded protein is a receptor on natural killer (NK) cells that interact with MHC class I (Webb *et al*., 2002). MDV is known to downregulate MHC class I, which adds to the intrigue.

The other two studies (McElroy *et al*., 2005; Heifetz *et al*., 2007) used commercial layer (egg-type) lines that allowed for larger populations and industrially relevant results. In both studies, individuals from two lines that differed in MD incidence were inter-mated to produce 5 BC families, and length of survival, which is correlated with MD incidence, was measured. Using microsatellites genotyped on DNA pools from selected individuals, McElroy *et al*. (2005) identified 17 markers out of the 81 screened as being associated with survival length. Heifetz *et al*. (2007) identified 15 QTL on two consecutive BC populations; however, only 5 of the QTL were common to both BC. The second BC hatch showed an MHC association, although the $B^{*}2$ allele was unexpectedly found to confer susceptibility. The interaction of the MHC with other 'background' genes has been observed previously (Hartmann, 1989).

Comparison of all four QTL scans for MD resistance indicates that those on chromosomes 2, 4, 7 and 8 are repeatedly found when only the significant ones are considered, and many more appear to be in common when suggestive levels are used.

However, the biological complexity of genetic resistance to MD needs to be emphasized. This challenge is revealed when the $6 \times 7 F_2$ MD resource population was re-evaluated using an additional 578 SNPs, and two-locus epistatic interactions were investigated (Cheng *et al*., 2007). A large number of highly significant (genome-wide $P \leq 0.001$) two-way interactions were found for viraemia but not for MD or survival. A single region on chromosome 1 accounted for loci in 166 of the 239 highly significant interactions. Nearly all of the interacting loci were not in the QTL regions previously identified. Therefore, interactions between loci can make a substantial contribution to variation in complex traits. Genome-wide QTL scans should be planned with future genotyping and analytical capabilities in mind; however, the challenge remains to identify the genes underlying the QTL.

To address the extreme difficulty in identifying positional candidate genes or tightly linked markers for MD resistance (or any QTL) using genome scan (top-down) approaches only, gene expression profiling using microarray technology (a bottom-up approach) has been integrated into MD resistance studies. The hope is that microarrays will identify genes and pathways involved in MD resistance, which combined with genetic mapping can reveal positional candidate genes (Liu *et al*., 2001a). In other words, positional candidate genes are those that have a genetic association and are identified as being relevant through gene expression analyses. Studies utilizing DNA microarrays have benefited greatly by technological advancement; current arrays contain all the potential open reading frames in the chicken genome and often also contain genes from pathogens, including MDV. This combination of host and pathogen genes can provide additional information on host–pathogen interactions.

Gene expression profiling has been conducted to identify differentially expressed genes between MD-resistant and MD-susceptible lines after MDV challenge (Liu *et al*., 2001a; Sarson *et al*., 2008), among B (MHC) congenic lines of chickens following inoculation with different MD vaccines (unpublished data), in chicken embryo fibroblasts (CEF) infected with MDV (Morgan *et al*., 2001) and in CEF transformed with Meq, the probable MDV oncogene (Levy *et al*., 2005).

A number of genes and pathways have been identified that are consistently associated with either MD resistance or MDV infection, and the results suggest that chickens with immune systems that are more stimulated by MDV infection are more susceptible. Because MDV is thought to only infect activated lymphocytes, chickens with immune systems that are more responsive may present more targets for MDV to infect and later transform.

Protein–MDV-chicken protein interactions may provide additional evidence that genes are associated with MD resistance. Niikura *et al*. (2004) screened for MDV-chicken protein–protein interactions using a two-hybrid screen, confirmed by an *in vitro* binding assay, and 9 MDV-chicken protein interactions were identified. Of particular interest were growth hormone (*GH1*; Liu *et al*., 2001b), stem cell antigen 2 (*SCA2*; Liu *et al*., 2003) and MHC class II β chain (*BLB*), as the transcripts for each gene were differentially expressed between MD-resistant and MD-susceptible birds following MDV infection, and there is an association to MD resistance. Also, *GH1* alleles have changed in response to selection for MD resistance (Kuhnlein *et al*., 1997), which supports growth hormone as an MD resistance gene. Novel upregulation of MHC class II following MDV infection (Niikura *et al*., 2007) and vitamin D receptor (*VDR*), which modulates MHC class II cell surface expression and is associated with MD resistance (Praslickova *et al*., 2008), further supports MHC class II β chain as a candidate gene for MD resistance.

Protein profiling using mass spectrometry, a powerful technique that can query the proteome, was conducted on UA-01 cells, these being MDV-transformed cells (Buza and Burgess, 2007). Prior work had indicated that MD tumours overexpress CD30 and, thus, might be a natural model for human T cell lymphomas (Burgess *et al*., 2004). Bioinformatic analyses of the data indicate that MD has a pattern that is consistent with other tumours, which probably originate from regulatory T cells. More importantly, the pro-metastatic integrin and ERK/MAPK signalling pathways were predominant, which suggests that these pathways are important for transformation and migration of MD tumours. Similar studies by Liu *et al*. (2006) have catalogued the spectrum of MDV proteins expressed.

Avian influenza

Avian influenza (AI) is a new or emerging disease problem. AI has been a disease of poultry since the 1870s although only sporadic outbreaks have occurred. However, the isolation of H5N1 high-pathogenic avian influenza (HPAI) that infected and killed 6 people in Hong Kong in 1997, along with more outbreaks associated with high mortality rates, has raised worldwide concern that HPAI might be the cause of the next flu pandemic (reviewed in Thomas and Noppenberger, 2007; Lee and Saif, 2008; Swayne and Halvorson, 2008). Thus, AI control is of wider importance as a zoonotic disease with significant human health concerns. Primary control of AI is through detection of infected animals; a single infected individual triggers eradication of the entire flock as well as those in the surrounding area. Thus, alternative control methods must either provide sterilizing immunity or prohibit viral transmission.

The *MX1* gene on chromosome 1, which encodes the myxovirus (influenza virus) resistance 1 protein, commonly referred to as Mx, has been the focus of several studies. Mx proteins belong to a family of GTPases that provide significant antiviral activity against AI infection in mice and many other animal species (Haller *et al*., 2007). Results on Mx antiviral activity and associations with AI resistance have been inconsistent. Ko *et al*. (2002, 2004) transfected Mx cDNA from chickens with different *MX1* alleles and found antiviral activity that was associated with an amino acid substitution at position 631 (Asn encodes antiviral activity while Ser had none). However, a wide variety of other studies in chickens and ducks, including *in vivo* and *in vitro* challenge studies and genetic studies, have failed to detect associations with the Mx gene (Bazzigher *et al*., 1993; Bernasconi *et al*., 1995; Li *et al*., 2006; Balkissoon *et al*., 2007; Kothlow and Kaspers, 2007; Livant *et al*., 2007; Watanabe, 2007; Benfield *et al*., 2008; Sironi *et al*., 2008).

There are at least two possible explanations for the disparity between reports that do or do not demonstrate antiviral activity to AI in chickens. First, strains of influenza vary in their response to mouse and human Mx proteins (Dittmann *et al*., 2008); thus, the strain used by Ko *et al*. (2002, 2004) is more sensitive to the antiviral activity of the Asn-containing chicken Mx protein. Also, results obtained from *in vitro* studies may often not reflect the actual situation in the live chicken (Sarmento *et al.,* 2008; Wu *et al.,* 2008). None the less, the observation that some chickens can survive following challenge with HPAI deserves further attention.

Finally, while not currently related to genetic improvement of disease resistance, it is worth mentioning novel efforts to control AI in poultry. Of particular interest is the potential for RNA interference (RNAi) to inhibit AI. RNAi is a mechanism whereby the highly conserved machinery of the cell is used to degrade or suppress the translation of mRNA in a sequence-specific manner. Since the process in animals is highly sequence driven, one can design vectors that target very specific transcripts, such as a particular viral mRNA. As RNAi has been successful as an antiviral reagent in the laboratory setting against many viruses in humans (see review by Haasnoot *et al*., 2007), it is logical to believe that RNAi targeting of AI might be useful as an adjunct control method. Currently, RNAi has shown some, albeit limited, success in inhibiting other avian viral pathogens, including MDV (Chen *et al*., 2008) and ALV (Chen *et al*., 2007); hence it is not surprising that many commercial companies as well as research groups are pursuing this option.

Other diseases

There are several other viral diseases of interest to the poultry industry. Infectious bursal disease (IBD), which is often referred to as Gumboro disease in reference to Gumboro, Delaware, where the first outbreak occurred, is a highly contagious viral infection. The causative agent is the infectious bursal disease virus (IBDV), a birnavirus, which contains a genome of two dsRNA segments that encode five viral proteins. As the name implies, IBDV targets the bursa of Fabricius, a key organ for the immune system, and is similar to MD in

being immunosuppressive. Vaccination is the primary control method, but the proper choice and timing of vaccination makes IBD control challenging.

Newcastle disease is another highly contagious disease, caused by the Newcastle disease virus (NDV), with symptoms that vary depending on the NDV pathotype. Lentogenetic NDV pathotypes do not cause disease in adult birds and are widely used as live vaccines. Mesogenic NDV pathotypes have intermediate virulence while velogenic pathotypes cause high mortality. NDV is a paramyxovirus with a non-segmented, negative sense ssRNA genome. The fact that NDV can affect all bird species and is zoonotic, velogenic pathotypes are endemic in many countries and virulent strains can mutate from low virulence to high virulence makes NDV and its control critical to the poultry industry.

Infectious laryngotracheitis (ILT) and infectious bronchitis (IB) are two acute respiratory diseases induced by the infectious laryngotracheitis virus (ILTV), a herpesvirus, and the infectious bronchitis virus (IBV), a coronavirus, respectively. Like most poultry diseases, ILT and IB are controlled through a combination of animal husbandry and vaccination.

Despite the economic importance of these diseases, studies to advance disease control reflect the constraints of resources on the poultry research community. Specifically, for the most part, all disease can be managed satisfactorily and the industry relies heavily on vaccination. Furthermore, since specific disease incidence ebbs and flows, there are relatively few long-term efforts to examine the genetic basis for differences in disease incidence. This is also reflected in the paucity of studies to incorporate genetic resistance to augment existing disease control measures.

This situation may and should change with the new tools that enable comprehensive analysis of individuals that vary in disease resistance. Thus far, for the diseases mentioned above, only DNA microarrays have been applied to the investigation of host responses and pathogenesis of infection. Specifically, Dar *et al*. (2005) used a custom cDNA microarray to examine responses in the lung tissue of 18-day-old chick embryos infected with IBDV at 6, 24, 48 and 72 h post-infection. Ruby *et al*. (2006) used a different custom cDNA microarray and applied it to investigate responses of bursas from IBDV-challenged chicks. Most relevant was the use of experimental lines that were resistant and susceptible to IBD. The results suggested that resistance to IBD was due to a more rapid inflammatory response and p53-related induction of apoptosis in the target B cells. These findings may provide candidate genes in the underlying pathways for future genetic testing.

Future Prospects

Given the pace of technological advancements that have occurred in the last few years, and the current funding in the biomedical community, it is safe to say that the way we conduct science and the information gleaned from its use will continue to grow rapidly. Already, the identification of copy number variation (CNV) and microRNAs that influence biological processes, including those that affect disease, is stimulating significant efforts that will probably provide

dividends in the future in terms of resolving the complexity of biology. Additionally, new sequencing platforms already in place and those yet to come are beginning to make impacts as well. Thus, with the genome assembly, existing and new technologies and more, the potential and possibilities for expanding our knowledge and the ability to apply it to the genetic improvement of chickens, including disease resistance, have never been greater.

Despite the excitement, with limitations on resources, academic scientists and the poultry industry must make judicious use of these technologies to maximize advancement. As Thomas Friedman stated in his book entitled *The World Is Flat*, '[i]ntroducing new technology alone is never enough. The big spurts in productivity come when a new technology is combined with new ways of doing business'.

Throughout this chapter and much of this book, genetic improvement normally implies genetic selection within commercial stocks. For enhanced genetic resistance to disease using MAS, this assumes that: (i) alleles that confer resistance are present; (ii) these alleles can be identified; and (iii) their frequency can be increased. The first assumption, i.e. the presence of resistant alleles in lines under selection, may be challenged by a recent biodiversity survey. Using a 3K SNP array and genotypes from 2580 individuals including 1440 commercial birds, it was determined that commercial chickens possess, at most, 50% of the genetic diversity found among all chicken breeds (Muir *et al*., 2008). And, at most, only ~10% of the missing diversity can be recovered by hypothetically combining all commercial stocks. This information suggests that the poultry industry might not have the genetic diversity to combat new and emerging diseases, especially in instances where the resistance is attributed to rare alleles, which might only be found in non-commercial breeds.

If this is the case, then the identification of novel disease resistance alleles will be essential, which addresses the second assumption. With technologies that can rapidly and accurately genotype thousands of birds for many thousands of genetic markers, we are already seeing that the rate-limiting step is obtaining phenotypes for each trait. For disease, this is even more problematic as there are often not very good or quantifiable measures of disease progression. Furthermore, screening of non-commercial breeds, especially indigenous breeds that survive under constant disease pressure, is difficult. Also, it is difficult to conduct controlled challenges for many viral pathogens. But with every challenge, opportunities for significant advancement are presented.

If significant resistance to a specific viral pathogen is found in a non-commercial bird, then the problem arises as to how to bring the causative allele into commercial populations. Introgression into elite commercial flocks is possible and can be accelerated using molecular markers. However, this is a time-consuming process that has never been employed. Alternatives include the direct modification of the commercial bird genomes. Scientifically, significant progress has been made recently with the ability to culture and modify primordial germ cells (PGCs) and retain their ability to form germ cells that can be transmitted to progeny (van de Lavoir *et al*., 2006). Thus, it is now possible to add

transgenes from any source including non-commercial birds, RNAi constructs or those conferring pathogen-derived resistance.

Finally, selection for enhanced disease resistance has been a topic for many years but normally is not paid serious attention until an outbreak occurs. At the same time, the reality of the industry forces and the industry's capacity for reactive responses, developments in genomics and the advancements in our biological knowledge argue for a more proactive role. The current expected increase in the world's population and wealth will contribute to higher demands for food that must be produced from ever less land available for farming, possibly hindered by potentially damaging environmental changes. These challenges mean that the need to integrate disciplines, especially genomics, immunology, veterinary sciences and nutrition, to address disease control as part of long-term solutions to issues in poultry production will grow with time.

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6 Bovine Viral Diseases: the Role of Host Genetics

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Summary

This chapter explores the major viral diseases of cattle and identifies those that are likely to be amenable to control assisted by host genetic selection, using either known polymorphisms in disease resistance genes or whole-genome approaches. Candidate genes for viral resistance include host receptors, pathogen recognition receptors, genes in the type I interferon pathway, restriction factors, cytokines, genes associated with viral and infected cell destruction, natural killer (NK) cell receptors and ligands, and genes in the major histocompatibility complex. Considerable evidence exists for the role of polymorphisms in genes in each of these categories in resistance to various viral diseases. However, attention is increasingly turning to the potential use of whole genome scans to find markers that may either be used to assist in the identification of causal mutations or to breed for resistance. Cattle viral diseases where host genetic variation has been quantified, or significant genetic marker associations demonstrated, include bovine leukaemia, various bovine respiratory diseases, bovine herpesvirus 1 (BHV-1) and, anecdotally in cattle, foot and mouth disease. Breed or species-level variation in resistance to rinderpest, malignant catarrhal fever (MCF) and lumpy skin disease has also been reported. Overwhelming evidence suggests that genetics could provide new approaches to disease control in the rapidly changing environment faced by cattle breeders, both in developed and developing countries. However, many obstacles and difficulties lie ahead if we are to achieve these goals, with bridging the phenotype gap and fully understanding the mechanisms of disease resistance being major challenges.

Introduction

In this chapter we will explore the major viral diseases of cattle from a local and a global perspective, focusing on those that are likely to be amenable to control through knowledge about polymorphisms in host disease resistance genes. We will review the evidence to date, and ask whether and how genetics and genomics of cattle hold promise for breeding approaches to viral disease control. We will discuss

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candidate genes, phenotypes and the gaps in knowledge, and the problems associated with collection of phenotypic trait data for viral diseases of cattle.

The scale of infectious disease in livestock continues to be a significant economic and welfare problem worldwide, even in well-managed systems. Farm animals remain at risk from endemic, exotic and newly emerging pathogens. With increasing global trade, climate change and loss of habitat, the issues surrounding biosecurity seem unlikely to diminish. Vaccination is often touted as the best possible route to infectious disease management, and yet for many pathogens either there are no appropriate vaccines or those that are available are far from ideal. Other control measures include good management and biosecurity, but in practice these can be difficult to maintain. Another route to control is eradication, which has proved successful (mainly) for certain viral diseases of cattle in some parts of the world, notably rinderpest and also, in some countries, foot-and-mouth disease virus (FMDV) (Grubman and Baxt, 2004) and bovine leukaemia virus (BLV) (Rhodes *et al*., 2003). However, within recent history there have been a number of incursions of exotic, highly infectious viral pathogens into the UK and Europe that have implications for cattle, e.g. FMDV (Saiz *et al*., 2002) and bluetongue virus (BTV) (Saegerman *et al*., 2008), and it seems unlikely that these incursions will be the last. For example, the capripox virus, lumpy skin disease virus (LSDV), has spread north and south from sub-Saharan Africa, and is now posing a threat to Asia and Europe (Babiuk *et al*., 2008). At its most extreme, global eradication requires cooperation from many countries over a long span of time to be successful. None the less, after 20 years of effort for the morbillivirus, rinderpest virus (RPV), eradication is almost complete worldwide (Normile, 2008). However, possible issues may yet arise as this situation has potential to leave a niche for another morbillivirus such as canine distemper virus or peste des petits ruminants to change host or habitat, and without vaccination or natural exposure cattle will be naive and thus highly susceptible. It is unclear whether sufficient political enthusiasm will be generated to pursue global eradication of any further viral pathogens of livestock in the future, although planning for progressive risk reduction has been advocated, for example, for FMDV (Rweyemamu *et al*., 2008).

Unlike the development of a few antiviral pharmaceuticals for human infections, there are no antivirals on the market for bovine viral infections. Thus breeding for resistance to viral infections could play an important role in disease control for livestock and, in the absence of viral evolution, would provide a sustainable and permanent solution which could be implemented for both large-scale producers (through artificial insemination (AI)) and small-scale subsistence farmers.

Viruses pose particular problems for the control of livestock disease because of their very nature: many are easily mutatable, e.g. FMDV (Domingo *et al*., 2005); they readily evolve to colonize new species, e.g. bat viruses, Nipah and Hendra paramyxoviruses, which are apparently non-pathogenic in their bat hosts, have recently jumped species into pigs and humans with devastating effects (Wong *et al*., 2002); and new emerging viral diseases of cattle are being reported such as bovine ephemeral fever caused by a rhabdovirus (Walker, 2005). The rapidity with which viruses can change would seem to far outweigh

the ability of hosts to evolve countermeasures or increase the frequency of resistant alleles in at-risk populations. In addition, viruses hide within host cells, making it more difficult for the host to detect and eliminate them without fatally damaging itself.

Despite these 'clever' strategies by viruses, host populations do become resistant, through co-evolutionary mechanisms. The genes involved in vertebrate host defence against viruses are thus the most likely to be rapidly evolving through changes in sequence, copy number variation as well as gene duplication and loss events, leading to changes in sizes of gene families (Hughes *et al*., 2005). There is some evidence that genes encoding components of innate immune pathways are particularly evolutionarily diverse (Lazarus *et al*., 2002), although the most polymorphic immune-related genes in the host-vertebrate genome are of course the classical genes of the major histocompatibility complex (MHC), which are involved in the initial presentation of pathogen antigens to the acquired immune system. However, MHC class I genes also play a key role in one aspect of innate immunity to intracellular pathogens such as viruses: recognition of 'altered self' by NK cells, which can be one of the main signs that a cell is infected with a virus (Bashirova *et al*., 2006). Consideration of these co-evolutionary strategies of host and virus can suggest potential candidate genes underlying host resistance to viral pathogens (see below).

The most important viral diseases of cattle at a global level are listed in Table 6.1, and those for which there is evidence of host genetic resistance are indicated. Unfortunately much of the information in this table is anecdotal, e.g. reported observations by veterinarians. In this chapter we will mainly concentrate on a few examples, as in reality few appropriate data have been collected. Although it is tempting to suggest that we now have the tools to identify genotypic variation, which is discussed elsewhere in this book, there is a major lack of information on phenotypic variation. Most evidence to date would suggest that host resistance is probably mediated by many genes, each of relatively small effect, and this means that large-scale studies are required to identify the causal genes. In addition, the phenotypic expression of host resistance to viral infection is often only manifest when challenged with the virus, and this in itself can lead to underestimation of heritability, which is often apparently low or non-existent. However, if this is the case, then selection may actually be more effective than predicted.

With the long generation time of cattle to take into consideration, as well as the expense of challenging large animals in a controlled manner, this has meant in practice that most genetic studies in cattle have been conducted in the field. This type of approach has resulted in successful selection indices for phenotypes measured routinely by industry, such as growth and milk yield. In contrast, the collection of field data in relation to resistance to viral disease has many limitations. This is partly because it is often not clear what the correlates of protection consist of, and therefore what parameters should be chosen as phenotypic traits, and this raises the issue of difficulties in measuring relevant factors. Since the observed variation in viral resistance has many components apart from any potential genetic influence, including dose of infection, previous exposure, age, sex, physiological condition, management, etc., this means that

Table 6.1. Major viruses currently a threat to cattle worldwide.

Virus	Genus/Family	Distribution	Control	Evidence for genetic susceptibilty
FMDV	*Aphthovirus/Picornaviridae* (linear, +ve sense, ssRNA)	Worldwide, except UK, EU, USA, Australia, New Zealand	Quarantine and slaughter in non-endemic countries; vaccination in endemic countries	Yes[a] but mainly anecdotal; species variability[b]
BVDV	*Pestivirus/Flaviviridae* (linear, +ve sense, ssRNA)	Worldwide	Vaccination	Not known
BLV	*Deltaretrovirus/Retroviridae* (diploid, linear, +ve sense ssRNA	Worldwide, except EU	Slaughter of infected animals	Yes: BoLA class II,[c] TNF,[d] alpha-lactalbumin[e]
BRSV	*Pneumovirus/Paramyxoviridae* (linear, –ve sense, ssRNA)	Worldwide	Vaccination	Analogy with human RSV
BPIV3	*Respirovirus/Paramyxoviridae* (linear, –ve sense, ssRNA)	Worldwide	Vaccination	Not known
RPV	*Morbillivirus/Paramyxoviridae* (linear, –ve sense, ssRNA)	Eradicated?	Vaccination	Yes: breed differences[f]
BHV-1	*Varicellovirus/Alphaherpesvirinae* (linear, dsDNA)	Worldwide	Vaccination	Yes: type I IFN[g]
BPV	*Delta-, Xi-, Epsilon-papillomavirus/Papillomaviridae* (circular, dsDNA)	Worldwide	None	Not known
BRV	*Rotavirus/Reoviridae* (11 dsRNA segments)	Worldwide	Vaccination	Not known

Continued

Table 6.1. Continued.

Virus	Genus/Family	Distribution	Control	Evidence for genetic susceptibilty
BTV	*Orbivirus/Reoviridae* (10 dsRNA segments)	Australia, USA, Africa, Middle East, Asia, Europe	Quarantine and slaughter; vaccination	Not known, but breed differences in sheep exist (anecdotal)
MCFV	Ovine herpesvirus -2; Alcelaphine herpesviruses *Rhadinovirus/Gammaherpesvirinae* (linear dsDNA)	Worldwide	Separating cattle from sheep and goats	Yes: MHC class II haplotypes in Bison[h]
Coronavirus	*Coronavirus/Coronaviridae* (linear +ve sense ssRNA)	Worldwide	Vaccination	Not known
VSV	Vesicular stomatitis virus *Vesiculovirus/Rhabdoviridae* (linear, –ve sense, ssRNA)	North and South America	Movement restriction and quarantine	Not known
Bovine ephemeral fever virus	*Ephemerovirus/Rhabdoviridae* (linear, –ve sense, ssRNA)	Africa, Australia, Asia	Vaccination	Not known; symptoms less severe in water buffalo than cattle
Rift valley fever virus	*Bunyaviridae/Phlebovirus* (3 –ve sense ssRNA segments)	Africa	Vaccination	Not known
LSDV	*Capripox, Poxviridae* (linear dsDNA)	Africa and Middle East	Vaccination; quarantine and slaughter	*Bos taurus* breeds, particularly Jersey, Guernsey and Ayrshire, are more susceptible to clinical disease than zebu cattle (*Bos indicus*) (anecdotal)

[a]Samina *et al.* (1998); [b]Alexandersen and Mowat (2005); Wernery *et al.* (2006); [c]Xu *et al.* (1993); Lewin *et al.* (1999); Udina *et al.* (2003); Juliarena *et al.* (2008); [d]Konnai *et al.* (2006); [e]Bojarojc-Nosowicz *et al.* (2008); [f]Pastoret *et al.* (2006); Wohlsein and Salik (2006); [g]Ryan *et al.* (1993); and [h]Traul *et al.* (2007).

even greater numbers of animals are required for field study than for experimental herds, where the environmental variables can be at least somewhat limited. Viral load would seem an important criterion, but of course this parameter changes over time, and thus field data can only measure an average, without the knowledge of when an animal was infected or whether viral load is increasing or decreasing. In addition, appropriate samples may be obtained only with some difficulty. Although death is one extreme outcome, most viral infections result in morbidity rather than mortality, and there may well be a range of clinical signs that relate to the degree of morbidity. Correlates of protection are often not known, or are complex to measure. Antibody is the simplest measure, as serum samples can be stored and investigated at leisure. However, even here, the most appropriate sample to measure the humoral response may not be circulating antibody but mucosal antibody at the site of infection. In addition, innate immunity parameters may only be detectable transiently, e.g. cytokines, and because of the intracellular nature of viruses, protective immunity is often not humoral but is mediated by T cells, which are considerably more difficult to assess. Thus, essentially there is a 'phenotype' gap that may continue to hinder application of genetic approaches to disease control.

In addition, the genes underlying resistance versus tolerance (where resistance refers to non-infection or mechanisms that reduce pathogen burden, and tolerance to maintenance of performance or reduced pathology, in the face of infection, where the mechanisms are not dependent on pathogen killing) may be very different (Schneider and Ayres, 2008). The added caveat is that no genes have yet been discovered that unequivocally control 'tolerance', as opposed to 'resistance'. In some cases it may be more appropriate to consider tolerance to a particular virus as the breeders' goal, whereas under other circumstances resistance may be more relevant. One possible solution to the phenotype gap may be to measure response to vaccination, as in many cases genes underlying variation in response to live pathogens may overlap with genes determining response to vaccination (Glass, 2004). None the less, this is not a complete solution, as acceptable vaccines should not induce pathology, unlike many live viruses, which would imply that genes underlying 'tolerance' or alleviation of disease severity might not be found by such an approach. In addition, for many viral diseases of cattle no vaccine exists, and of those that do exist, only a limited range is given routinely.

As discussed elsewhere in this book, investigation into genes underlying complex disease resistance/susceptibility can take complementary approaches: candidate genes deduced from knowledge about the cellular pathways leading to protection or pathology, or whole genome scans using markers spread across the genome. Very few genome scans for disease resistance traits in cattle have been published, with none for viral diseases, and all have involved a limited number of microsatellite markers, which effectively means that the quantitative trait loci (QTL) revealed have large confidence intervals making it difficult to identify causal genes. However, it is now possible to conduct whole-genome studies in cattle using a commercially available single nucleotide polymorphism (SNP) chip for cattle which contains over 50,000 SNPs (http://www.illumina.com/downloads/BovineSNP50_data_sheet.pdf) and was developed

through a de novo SNP discovery project (Matukumalli *et al*., 2009) using information derived from the recently sequenced bovine genome (The Bovine Genome Sequencing and Analysis Consortium, 2009). Exploitation of this SNP chip is being undertaken in cattle in relation to bovine respiratory disease (BRD) (http://www.ars.usda.gov/is/pr/2008/080805.htm). The 50K SNP chip currently available, or even denser chips, may provide sufficient coverage of the bovine genome, so that researchers could combine their data and samples from across different populations, and it may be possible to retrospectively undertake such an endeavour with an SNP panel, if sufficient DNA samples of requisite quality from cattle with suitable phenotypes have been collected.

Candidate Genes for Resistance to Viruses

In terms of candidates, there are many possibilities. One of the most fruitful ways to identify such genes may be to consider the early stages of host–pathogen interactions, and particularly those pathways that the virus targets for host evasion, as the host is likely to evolve new ways to limit the effects of viral invasion, resulting in genetic diversity. Such selective pressure on host defence genes may be detectable as so-called signatures of selection, and genome-wide analysis has identified that positively selected genes in mammals are indeed enriched for host defence genes and show more evidence for episodes of positive selection than other parts of the genome (Kosiol *et al*., 2008). Table 6.2 comprises a non-exhaustive list of likely candidate genes for resistance to viruses, together with their chromosomal location and reported polymorphisms, as well as evidence for involvement in the genetics of resistance to viral infection in cattle. However, as can be seen, very few candidate gene polymorphisms have been shown to play a role in resistance to viral infection in cattle, and none has been analysed quantitatively in terms of their contribution to observed phenotypic variation. The host cell type invaded depends on the virus, with some viruses preferentially inhabiting non-immune cells and others deliberately targeting the very immune cells that form part of the host defence, where they can directly subvert the immune response. The type of cell invaded will thus have different arsenals to attack the viral invader, with immune cells expressing the widest range of receptors and other factors that recognize, signal alarm, damage and destroy viruses.

Host receptors

First, viruses have to find their way into cells that can support their replication. Thus it is feasible that some animals could be completely resistant to infection because they lack appropriate viral receptors. A prime example of this is in the human field, where the chemokine receptor mutation, CCR5Δ32, which contains a stop codon inhibiting expression of the receptor on the cell surface, prevents homozygous individuals becoming infected with human immunodeficiency virus (HIV), and slows progression of the infection in heterozygotes (Dean *et al*., 1996). However, the cellular receptors used by the main viruses

Table 6.2. Candidate genes and loci for resistance/susceptibility to viral infections in cattle.

Candidate gene	Role in host–viral interactions	Evidence for involvement in viral defence in cattle	Chromosome location in cattle: BTA	Polymorphism in cattle	Genetic association with resistance and susceptibility to viral infections in cattle
αvβ6 integrin receptor (ITGAV and ITGB6)	Uptake of FMDV[1]	FMDV receptor[1]	2[2]	NR	NR
CD46	Uptake of BVDV[3]	BVDV receptor[3]	16[2]	NR	NR
CD150	Uptake of RPV[4]	RPV receptor[4]	3[2]	NR	NR
FcγRI (CD64), FcγRII (CD32) and FcγRIII (CD16)	Uptake of viruses coated with IgG; ADCC	? FMDV[5] and probably other viruses	3[6]	NR	NR
FCαR (CD89)	As above with IgA	NR	18[7]	NR	NR
FcRn	Neonatal levels of IgG	NR	18[8]	Yes[8]	NR
TLR3	PRR: ssRNA	BVDV[9]; BRV[10]	27[11]	yes[12]	NR
TLR4	PRR: viral ligands	BRSV[13]; FMDV[14]	8[15]	Yes[15]	NR
TLR7	PRR: dsRNA	BVDV[9]	X[11]	Yes[12]	NR
TLR8	PRR: dsRNA	NR	X[11]	Yes[12]	NR
TLR9	PRR: CpG dsRNA	NR	22[16]	Yes[12]	NR
AIM2	PRR: dsDNA; cell death	NR	3[2]	NR	NR
RIG1	PRR: viral RNA	BVDV[17]	8[18]	Yes, one only	NR
NOD2	PRR: viral ligand; cell death	NR	18[19]	Yes[19]	NR
MAVS	Antiviral signalling	BVDV[17]	13[19]	NR	NR

Continued

Table 6.2. Continued.

Candidate gene	Role in host–viral interactions	Evidence for involvement in viral defence in cattle	Chromosome location in cattle: BTA	Polymorphism in cattle	Genetic association with resistance and susceptibility to viral infections in cattle
IRF3	Antiviral signalling; transcription factor	BVDV[17]; BRV[20]	18[21]	NR	NR
IFN-α and type I IFN	Major innate antiviral defence	Several; BRSV,[22] BVDV[23] and BHV-1[23] antagonize	8[24]	Yes[25]	BHV-1[25]
MX	Inhibition of viral transcription	BHV-1[26]; BRV[26];	1[27]	Yes[28]	NR
Oas 1	RNA decay; flavivirus resistance	NR	17[29]	NR	NR
PKR	PRR: activated by dsRNA; inhibition of viral transcription	FMDV[30]; BVDV[17;31]	11[32]	NR	NR
APOBEC	Restriction of retrovirus	NR	5[2]	NR	NR
TNFα	Apoptosis and inflammation	Role in many viral infections	23[33]	Yes[33]	BLV progression[34]
IFNγ	NK/CD8$^+$ T; non-cytopathic clearance of virus	Role in many viral infections	5[35]	Yes[35]	Higher[36] expression associated with BLV resistance (sheep)
IL-8	Pathology in RSV	Increased expression in BRSV infections[37]	6[38]	Yes[39]	NR

IL-10	Innate immune regulator; pathology in RSV	BLV[40]	16[41]	Yes[41]	NR
IL-12 p35 and p40	Inducer of Th1 responses	BLV[40]	1 (p35)[42] and 7[43] (p40)	Yes[42] (p35 only)	NR
Beclin-1	Autophagy	NR	19[2]	NR	NR
KIR	NK receptors	NR	18[44]	Yes[45]	NR
Ly-49 like	NK receptor	NR	5[44]	Yes[46]	NR
MHC class I	Antigen presentation; altered self; NK recognition	$CD8^+$ T cells primed by BPIV3[47]; BHV-1[48]; FMDV[49] and BRSV[50]; FMDV,[51] BPV[52] and BVDV[53] down-regulate	23[54]	Yes[54]	NR
MHC class II	Antigen presentation	BRSV[55]; FMDV[56]; BVDV[57]	23[54]	Yes[54]	BLV[58]; FMDV[59]
Non-classical MHC I	NK ligands	NR	23[60] and others	Yes[60]	NR
ULBPs	NK ligands	Not reported, but BHV-4 encodes a decoy[61]	9[61]	NR	NR

BTA, *Bos taurus*. NR, not reported.

[1]Monaghan *et al*. (2005); [2]based on latest build of the bovine genome (Btau_4.0); [3]Maurer *et al*. (2004); [4]Baron (2005); [5]Summerfield *et al*. (2009); [6]Klungland *et al*. (1997); [7]Morton *et al*. (2004); [8]Laegreid *et al*. (2002); [9]Lee *et al*. (2008); [10]Hodgson *et al*. (2005); [11]McGuire *et al*. (2006); [12]Cargill and Womack (2007); [13]Lizundia *et al*. (2008); [14]Zhang *et al*. (2006); [15]White *et al*. (2003); [16]Goldammer *et al*. (2004); [17]Shoemaker *et al*. (2009); [18]Cargill *et al*. (2006); [19]Taylor *et al*. (2006); [20]Pina-Vazquez *et al*. (2007); [21]Brunner *et al*. (2003); [22]Valarcher *et al*. (2003); [23]Randall and Goodbourn (2008); [24]Walker *et al*. (2009); [25]Ryan *et al*. (1993); [26]Muller-Doblies *et al*. (2002); [27]Ellinwood *et al*. (1999); [28]Nakatsu *et al*. (2004); [29]Perelygin *et al*. (2005); [30]Chinsangaram *et al*. (2001); [31]Gil *et al*. (2006); [32]Perelygin *et al*. (2006b); [33]Agaba *et al*. (1996); [34]Konnai *et al*. (2006); [35]Grosse *et al*. (1999); Schmidt *et al*. (2002); [36]Usui *et al*. (2007); [37]L. Hibbert, J.-F. Valarcher and G. Taylor (unpublished observations); [38]Modi *et al*. (1998); [39]Heaton *et al*. (2001); Leyva-Baca *et al*. (2007); [40]Kabeya *et al*. (2001); [41]Beever *et al*. (1997); [42]Schmidt *et al*. (2000); [43]Sonstegard *et al*. (2000); [44]Storset *et al*. (2003); [45]Dobromylskyj and Ellis (2007); Guethlein *et al*. (2007); [46]Birch and Ellis (2007); Fikri *et al*. (2007); [47]Bamford *et al*. (1995); [48]Hegde *et al*. (1999); [49]Guzman *et al*. (2008); [50]Gaddum *et al*. (1996); [51]Sanz-Parra *et al*. (1998); [52]Araibi *et al*. (2004); [53]Lee *et al*. (2009); [54]Lewin *et al*. (1999); [55]Fogg *et al*. (2001); [56]Gerner *et al*. (2009); [57]Collen *et al*. (2002); [58]Xu *et al*. (1993); Lewin *et al*. (1999); Udina *et al*. (2003); Juliarena *et al*. (2008); [59]Garcia-Briones *et al*. (2001); [60]Birch *et al*. (2008a,b); [61]Larson *et al*. (2006).

of cattle are mostly unknown, the exceptions being those for FMDV (αv integrins with αvβ6 receptor as the principle *in vivo* relevant receptor (Monaghan *et al*., 2005)), bovine viral diarrhoea virus (BVDV) (CD46, a member of the complement regulatory receptors (Maurer *et al*., 2004)) and RPV (signalling lymphocyte activation molecule (SLAM) or CD150 (Baron, 2005)), but even in these cases, it is likely that other receptors are involved. For example, cells of the innate immune system, including macrophages and dendritic cells (DC) can also become infected with viruses such as FMDV, and may do so through other receptor-mediated uptake, such as Fc receptors (Summerfield *et al*., 2009). There are several Fc receptors in cattle and other species that are expressed on a variety of different cell types, particularly myeloid cells and NK cells, and play a role in linking humoral immunity with cell-mediated responses (Kacskovics, 2004). Several of these have been mapped in cattle including *FcγRI*, *FcγRII* and *FcγRIII* to BTA3 (Klungland *et al*., 1997), the polymeric Ig receptor to BTA 16 (Kulseth *et al*., 1994), the *FCαR* to BTA18, flanking the NK *killer immunoglobulin-like receptor* (*KIR*) cluster (see below) (Morton *et al*., 2004) and the neonatal Fc receptor (*FcRn*) also to BTA 18 (Laegreid *et al*., 2002). However, polymorphisms have been reported in only the polymeric Ig receptor (Kulseth *et al*., 1994) and *FcRn* (Laegreid *et al*., 2002). Variation in the latter has been associated with differences in serum IgG concentration in newborn calves (Laegreid *et al*., 2002) and it is possible that such polymorphisms may impact upon viral diseases of calves. We have observed that breed-cross influences the level of circulating IgG to bovine respiratory syncytial virus (BRSV) in young calves prior to vaccination which, together with an indication that this may be heritable (O'Neill *et al*., 2006), suggests that genetic factors may influence the rate of maternal antibody clearance and therefore protection against BRSV infection; possibly this may be related to *FcRn* polymorphisms.

Pathogen recognition receptors

Second, the mammalian host innate defences are organized to recognize and signal alarm to the immune system through sets of receptors and molecules that recognize pathogen- or danger-associated molecular patterns (PAMPS or DAMPS) (Lotze *et al*., 2007). The former of these pattern recognition receptors (PRRs) recognize evolutionarily conserved molecules that form essential components of pathogens (PAMPs), whereas DAMPS are evolutionarily conserved host sequences associated with damage or inflammatory signals such as the expression of stress molecules on the surface of virally infected cells, or cytokines, such as the interferons, which inhibit viral growth. PRRs are mainly expressed in cells of the innate immune system, particularly macrophages and DC, as well as in cells that comprise barriers to infection such as skin epithelial cells and cells of the mucosal system (Zarember and Godowski, 2002). Thus for viruses, examples of PRRs include the intracellular membrane-bound Toll-like receptors (TLRs), e.g. TLR3, which recognizes single-stranded RNA; TLR7 and TLR8, which recognize double-stranded (ds) RNA; and TLR9, which

recognizes CpG motifs in dsDNA. All ten TLRs have been mapped in cattle (White *et al*., 2003; Goldammer *et al*., 2004; McGuire *et al*., 2006) (Table 6.2). Although TLRs are under strong purifying selection, TLRs in cattle (and other species) none the less show evidence of positive selection and polymorphism, particularly in their ligand-binding sites (White *et al*., 2003; Jann *et al*., 2008; Seabury *et al*., 2008; Werling *et al*., 2009), and SNPs and indels have been reported for the viral recognition TLRs (Cargill and Womack, 2007). ssRNA viruses of cattle such as FMDV, BVDV and the double-stranded DNA virus, bovine herpes virus (BHV)-1, modulate the expression of TLRs (Hodgson *et al*., 2005; Zhang *et al*., 2006; Lee *et al*., 2008), and BRSV shows evidence of interacting with TLR4 (Lizundia *et al*., 2008).

Other families of PRRs expressed within cells are beginning to be identified, and are likely to be particularly relevant for viral infections. They include the retinoic acid-inducible gene-I (RIG-I)-like receptor family that recognizes viral RNA as well as several PRRs that bind viral DNA (for a review see Bowie and Unterholzner, 2008), but their role is only beginning to be explored in viral infections of cattle, e.g. BVDV (Shoemaker *et al*., 2009). Very recently PRRs that sense cytoplasmic dsDNA were identified (Roberts *et al*., 2009). AIM2 (absent in melanoma 2) was shown to interact with cytosolic DNA leading to cell death, whereas p202 inhibits this pathway. These are members of the haematopoietic interferon-inducible nuclear protein (HIN) family, many of which contain N-terminal pyrin domains, which can potentially interact with inflammasomes and caspases to induce rapid cell death. Bovine *RIG-1* and mitochondrial antiviral signalling (*MAV*) genes have been mapped to BTA08 and BTA13 respectively (Table 6.2), but a limited study detected only a single intronic SNP in *RIG-1* (Cargill *et al*., 2006). Many of these PRRs induce intracellular signalling cascades involving many intermediate molecules such as MyD88, which ultimately activate transcription factors such as NF-kB as well as interferon response factor (IRF) family members to switch on type I interferons and inflammatory cytokines (Bowie and Unterholzner, 2008). We have recently identified *TLR1*, *TLR6*, *MyD88* and *IRF3* as the most likely candidates underlying a number of immune-related QTL in cattle and other livestock species (Jann *et al*., 2009). In the context of resistance to viral infections, IRF3 is particularly interesting as a candidate as it is one of the master switches for type I interferon synthesis and is the target for evasion of the host response by at least two viruses that infect cattle: BVDV (Shoemaker *et al*., 2009) and bovine rotavirus (BRV) (Pina-Vazquez *et al*., 2007). Another family of intracellular PRRs expressed in the cytosol are the nucleotide oligomerization domain (NOD)-like receptors (NLRs), some of which may sense viral infections (Takeuchi and Akira, 2009). NLRs form part of large macromolecular complexes, inflammasomes, which activate caspases, leading to activation of pro-interleukin 1 (IL-1) and pro-interleukin-18, as well as cell death. NOD2, which is also known as caspase recruitment domain 15 (*CARD 15*), acts as a PRR and is expressed in macrophages. In cattle *CARD15* maps to BTA18, and genetic variation has been described (Taylor *et al*., 2006). Pant *et al*. (2007) reported that a single SNP in the coding region of *CARD15* was associated with a mastitis-related trait, but no associations with viral disease have been reported so far.

Type I interferon pathway

Binding of PRRs with their ligands rapidly induces what is probably the major vertebrate innate defence mechanism against viruses, i.e. the production of type I interferons (IFN-α and IFN-β) (McCartney and Colonna, 2009). Not unsurprisingly, many viruses antagonize components of this pathway, including several viral pathogens of cattle, e.g. BRSV, BVDV and BHV-1 (Randall and Goodbourn, 2008). Thus the genes encoding molecules comprising the signalling pathways that switch on this response and the downstream effector molecules are also potentially important candidates. These include the type I IFNs themselves as well as interferon-inducible genes (*ISG*s or *IFN*-stimulated genes) such as the family of *2'-5' oligoadenylate synthase* (*Oas*) genes, *ribonuclease L* (*RNase L*) gene, *protein kinase R* (*PKR* or *Eukaryotic translation initiation factor 2-alpha kinase 2*) and *Myxovirus resistance* (*MX*) genes. Polymorphisms in some of these genes in mice and humans have been associated with resistance to viral infections (Sadler and Williams, 2008). The bovine type I interferons consist of two sub-clusters of over 50 genes on BTA08, with evidence for novel expansions and deletions in distinct families (Walker *et al*., 2009). Many of them have been shown to be polymorphic, with some associated with severity of BHV-1 infections in cattle (Ryan *et al*., 1993). *Mx* family genes are induced in cattle in response to several viruses (BHV-1 and BRV) and type I interferons (Muller-Doblies *et al*., 2002) and are polymorphic (Nakatsu *et al*., 2004). Many bovine viruses are susceptible to the effects of type I IFNs including FMDV, which may evade the innate immune response partly by shutting off this response in epithelial cells, though DC may be able to overcome this inhibition (Summerfield *et al*., 2009). However, other bovine viruses, including the single-stranded RNA paramyxoviridae, are resistant to the effects of bovine Mx (LeRoy *et al*., 2005). FMDV is also susceptible to the effects of PKR (Chinsangaram *et al*., 2001) and non-cytopathic BVDV appears to have developed mechanisms to inhibit PKR activity (Gil *et al*., 2006). There is considerable between-species diversity in PKR sequences, with evidence of positive selection probably driven by virus-encoded mimics of the PKR ligand, which may also help explain the limited host range of some viruses that need to produce dsRNA (Rothenburg *et al*., 2008). Oas1, which is also activated by the presence of dsRNA with polymorphisms associated with flavivirus resistance in mice, has undergone gene duplication events in cattle with a cluster of at least five loci on BTA17 (Perelygin *et al*., 2005, 2006a). Some evidence for polymorphisms in some of these *ISG*s has been described in cattle (Ryan and Womack, 1993; Nakatsu *et al*., 2004), but no correlations with viral resistance in cattle have been described so far.

In recent years there has been much interest in the potential role of RNA silencing as an antiviral defence mechanism. It is clear that in plants and invertebrates this is a major player, but the role of host microRNAs (miRNAs) in viral defence in mammals is less clear (Mahajan *et al*., 2009). It has been reported that specific host-dervied miRNAs are produced in response to activation of the type I interferon pathway, and these may regulate the host response and/or

target viral RNA for degradation. Expression of conserved and novel miRNAs from immune tissue in cattle has been reported (Coutinho *et al*., 2007; The Bovine Genome Sequencing Consortium, 2009), and thus polymorphisms in these regions may affect the bovine response to viral infections.

Restriction factors

Vertebrate cells infected with retroviruses also produce anti-retroviral proteins, including the *APOBEC* (apolipoprotein B mRNA-editing enzyme, catalytic polypeptide) cytodine deaminase gene (*A3*) family, which has undergone expansion and strong positive selection (Conticello *et al*., 2005). These so-called restriction factors can also target non-retroviral viruses, and there are probably many more to be discovered (Wolf and Goff, 2008). Sheep and cattle have three *A3* genes with the potential to code for a fourth A3 protein (LaRue *et al*., 2008), and these may play a role in resistance to BLV.

Cytokines

In addition to the type I interferons and their downstream molecules, viral infection may also induce other cytokines such as tumour necrosis factor α (TNF-α) produced by activated immune cells such as macrophages, and IFN-γ produced by NK cells and cytotoxic $CD8^+$ T lymphocytes (CTL) (Guidotti and Chisari, 2000). IFN-γ and other less well-characterized cytokines are likely to be involved in clearance of viruses from infected cells by non-cytopathic mechanisms. Signalling by TNF-α superfamily members, on the other hand, can also signal to both non-apoptotic and apoptotic pathways (Benedict, 2003), and viruses target almost every aspect of these pathways. In addition, polymorphisms in the cytokines associated with Th1 (particularly IL-12 and IFN-γ) and Th2 responses (particularly IL-10) may affect responses to viruses as these cytokines are pivotal in modulating and controlling pathways that determine the balance between pathology and protection. Large DNA viruses, in particular, contain many immunomodulatory genes, many clearly hijacked from their mammalian hosts. For example, cowpox viruses encode several viral homologues of components in the TNF-α and IFN pathways (Seet *et al*., 2003). Polymorphisms for a range of cytokines, chemokines and their receptors have been described for cattle and their positions mapped (Table 6.2; reviewed in Glass, 2004). Of these, only *TNF-α* variants have been associated with resistance to viral disease in cattle – progression of BLV infection (Konnai *et al*., 2006) (see below). TNF-α and IL-8 and IL-10 polymorphisms in humans have been associated with increased risk of human respiratory syncytial virus (HRSV) bronchiolitis (see below) and thus might play a role in variation in response to BRSV infections in calves. A more comprehensive exploration of polymorphisms in bovine cytokine and related genes and their relation to resistance to viral infection is clearly warranted.

Viral and infected cell destruction: autophagy and apoptosis

Eukaryotic host cell death occurs during normal and pathological processes as well as in response to microbial infection, and involves a number of different mechanisms (Fink and Cookson, 2005). Virally infected host cells have at least two evolutionarily conserved pathways that directly target the virus or the infected cell for destruction and thus directly limit viral replication and spread. Removal of infected cells can occur through programmed cell death or apoptosis, a non-inflammatory process. In addition, apoptotic cell uptake by effector cells such as macrophages and DC can trigger downstream effector mechanisms enhancing viral clearance (Peng *et al*., 2007). There are two distinct pathways that lead to apoptosis: extrinsic, which is mediated through ligation of death receptors of the TNF family; and intrinsic, which involves perturbation of mitochondria. Both pathways activate caspases, ultimately leading to apoptosis. The caspase family and other members of these pathways are targets of viral evasion mechanisms (Best, 2008), and thus may be considered potential candidates for disease resistance genes, although no evidence has been found to date in cattle.

Autophagy is an ancient evolutionarily conserved pathway through which cells can sequester, recycle and degrade cellular components through the specialized membrane-bound organelles called autophagosomes, which then fuse with lysosomes. Very recently evidence has suggested that this mechanism also plays an important role in cell defence against viral invaders themselves (Orvedahl and Levine, 2008). In this role, autophagy shares many of the same components of the PRR pathways described earlier, including the IFN-α-inducible gene, PKR. Although IFN-γ is a major trigger for autophagy, there appears to be considerable cross talk between all of these cell pathways as there is evidence that they can stimulate and inhibit each other. Autophagy also feeds pathogen-associated antigens from the cytosol into the antigen presentation pathways within the lysosome and thus links to the development of acquired immunity (see below). Although there are many details still being discovered about the components and importance of autophagy in viral defence, evidence is emerging that some viruses have developed mechanisms to subvert autophagy (Orvedahl and Levine, 2008). So far, one example of relevance to cattle has suggested that the morphological features in bovine cells shortly after infection with BVDV are reminiscent of autophagy (Birk *et al*., 2008). Some of the unique components of autophagy were first identified in yeast and more recently in vertebrates and include a series of autophagy-related genes (Atg) (Virgin and Levine, 2009). Beclin-1 (or *Atg-6*), which has antiviral function and is one of the most studied, is targeted by many different types of virus. So far mutations affecting autophagy-related genes have been mainly associated with Crohn's, the chronic inflammatory disease, in humans, but one polymorphism has also been linked to impaired intracellular pathogen clearance (Kuballa *et al*., 2008), suggesting that autophagy-related genes may be important candidate genes for viral resistance in cattle.

In summary, the host infected cell has a wide arsenal of factors and defence mechanisms aimed at overcoming a viral invader. These thus become targets of

the virus, which seeks to evade them, and through co-evolution the host will develop new strategies to circumvent the viral assault on the host defence pathways within the infected cell.

However, the vertebrate host has a further set of weapons: host immune effector cells. Probably the major immune effector cells involved in viral defence are NK cells and CTL. Both cell types are triggered through interactions with MHC class I molecules, although the nature of the interactions is rather different (see below).

NK cells, their receptors and ligands

Natural killer cells function as surveillance cells checking the integrity of host cells, and play an important role in viral defence, by lysing infected cells and also producing the main source of interferon-γ (IFN-γ) in the early stages of viral infection (Biron *et al*., 1999). NK cells express a very heterogeneous array of polymorphic inhibitory and activatory receptors (NKRs), which are encoded by two distinct families that map to different regions of the genome (Parham, 2008). Some of the activatory forms recognize 'altered self' MHC classical class I molecules, the nature of which is not entirely clear, but is associated with viral infection, and trigger NK activity, whereas the inhibitory forms recognize normal self MHC class I and thus spare the cells from destruction by NK cells (Bashirova *et al*., 2006). However, several NKRs recognize other molecules that have altered expression on cells infected with viruses. One hypothesis for the existence of these diverse receptor families is that they evolved to overcome the propensity for viruses to downregulate or otherwise interfere with the MHC class I processing and presentation pathways (see below). In support of this was the discovery that the underlying gene for resistance to murine cytomegalovirus (MCMV) is encoded by an NK activatory receptor that appears to have evolved to recognize an MHC class I-like 'decoy' molecule encoded by the virus (Arase and Lanier, 2002).

Two major groups of NKRs have been described. One family comprising the *KIR* genes is found in the leukocyte receptor cluster on BTA18 and has been expanded in the cattle genome (Storset *et al*., 2003). The leukocyte receptor cluster includes other *KIR*-like genes, such as *NKp46*, which appear to be expressed in all NK cells including cattle (Storset *et al*., 2003), as well as the two adapter molecules, DAP10 and DAP12 (Fikri *et al*., 2007). Interestingly, all but one of the cattle *KIR* genes appear to have evolved from a different lineage to that expanded in primates and humans (Guethlein *et al*., 2007). Analogous to the MHC class I genes in cattle, the *KIR* family is clearly complex, polymorphic and may have different numbers of genes per haplotype (Dobromylskyj and Ellis, 2007; Guethlein *et al*., 2007). NK cells also express a different set of NKRs, the C-type lectin superfamily, which maps to the NK complex on BTA5 in cattle (Storset *et al*., 2003). This region has been expanded in rodents, but not in cattle, although there is clearly diversity of genes and alleles in this bovine cluster as well (Birch and Ellis, 2007; Fikri *et al*., 2007).

As discussed earlier, NK cells recognize virally infected cells through changes in expression of MHC class I genes. However, many of the known NKR ligands are encoded by MHC class I-related non-classical genes. In cattle some of these are encoded within the MHC region, including the MHC class I chain-related (*MIC*) genes (Birch *et al*., 2008a), as well as several others that are also polymorphic (Birch *et al*., 2008b). In addition, there are other MHC class I-like genes in cattle that may also encode NK cell ligands, in other parts of the genome, including a diverse and positively selected family of genes, the UL16-binding proteins (*ULBP*s) localized in two clusters on BTA9 (Larson *et al*., 2006). No functional data yet exist for the interaction of any NK cell receptor and viral pathogens in cattle, but given the diversity of MHC class I and class I-like genes in the bovine genome and the fact that several bovine viruses down regulate MHC class I (Table 6.2), it would seem likely that both the NKRs and their ligands play an important role in viral defence in cattle, and their polymorphisms are likely to have evolved to overcome viral infections. Some support for this is cited by Larson *et al*. (2006), who noted that an open reading frame (ORF) of bovine herpes virus-4 (BHV-4) codes for a protein with 22% identity with the human CMV MHC class I 'decoy' protein, UL16.

Major histocompatibility complex

When examining the possibility of breeding cattle for enhanced resistance to viral diseases, the genes of the bovine *MHC*, or bovine leukocyte antigens (*BoLA*), are perhaps the ultimate candidates, as the MHC classical gene products are both central to the initiation of immune responses and probably the most polymorphic genes in the genome (Lewin *et al*., 1999). The *BoLA* locus on BTA23, spanning approximately 2.5 Mb, contains 154 predicted genes (The Bovine Genome Sequencing Consortium, 2009), with many having immunological functions (Brinkmeyer-Langford *et al*., 2009). These 154 genes can be subdivided, depending on function, into three distinct regions: I, II and III. Classical class I and II gene products are key molecules for initiating an adaptive immune response to pathogens such as viruses, as they present self peptides and foreign pathogen-derived peptides to T cells. MHC class I molecules have an additional role in that they are markers of self and are expressed on all cells, whereas MHC class II molecules are expressed only on antigen-presenting cells, including DC, myeloid cells and B cells, and (depending on the species, which includes cattle) following inflammatory signals on other cells such as T cells (Glass and Spooner, 1990). As described earlier, MHC class I expression on the cell surface is constantly monitored by NK cells, as part of host surveillance against invading pathogens. However, the role for which they are mainly known is antigen presentation, which links innate immunity to the cells of the acquired immune system. The polymorphisms are mainly located in the anchor pockets within the peptide-binding cleft (PBC) of classical class I and II molecules. These polymorphisms influence the affinity of binding of peptides, and thus determine the range of peptides available for T cell recognition and the magnitude of the ensuing immune response. Class I molecules

present endogenous peptides of between eight and ten amino acids in length to CTL, whereas class II molecules mainly present exogenous peptides of varying lengths to $CD4^+$ T cells. Presentation of endogenous self peptides is essential to maintaining tolerance to self, whereas endogenous foreign peptides are derived mainly from intracellular pathogens such as viruses. Induction of autophagy in virally infected cells (see above) can also deliver endogenously derived peptides into the MHC class II pathway, a process known as 'cross-presentation' (Guermonprez and Amigorena, 2005). Although viruses usually spend the majority of their life cycle in the intracellular milieu, many viruses are in fact found in the extracellular spaces, and thus peptides derived from viruses can also enter the MHC class II processing pathways in antigen-presenting cells such as macrophages and DC. In addition, in the specialized antigen-presenting cell, DC, peptides derived from extracellular sources can also bind and be presented by MHC class I molecules – another form of cross-presentation (Guermonprez and Amigorena, 2005).

MHC class I

MHC class I molecules are intimately linked with the cell machinery for recycling misfolded proteins, and are expressed only on the cell surface in association with endogenous peptides derived from the proteosome. Inflammation and intracellular infection both disrupt this pathway, leading to changes in MHC class I expression. The presence of MHC class I molecules on all cells is monitored by NK cells, which will lyse cells with 'altered' MHC class I expression (Bashirova *et al*., 2006). Clearly, therefore, MHC class I polymorphisms are particularly relevant for both the innate and acquired immune response to viruses.

The extreme polymorphisms in MHC loci are probably maintained by natural balancing selection, related to susceptibility and resistance to pathogens at the population level, with some evidence for overdominance and heterozygote advantage, though rare-allele advantage may also explain the polymorphism in MHC genes (Hughes and Yeager, 1998; Martin and Carrington, 2005).

It appears that the *BoLA* class I region is under strong selective pressure, which has led to considerable diversity, with up to six MHC class I loci that are expressed in different combinations depending on haplotype (Ellis, 2004), and there is evidence for interlocus recombination (Holmes *et al*., 2003). In addition, *BoLA* class I genes are highly polymorphic (see http://www.ebi.ac.uk/ipd/mhc/bola/ for a list of sequences), although the extent of the allele diversity is unknown (Babiuk *et al*., 2007). Clearly, increasing the number of different class I molecules expressed by the host should increase the range of peptides that can then be displayed to T cells, which suggests that cattle with additional loci and maximum heterozygosity at these loci might be generally more resistant to pathogens.

However, the associations between *BoLA* class I gene polymorphisms and resistance to viral infection has not been explored much, partly because of the technical difficulties in determining the number of loci per haplotype and typing of alleles, but also because large study sizes are required to provide convincing statistical associations with such polymorphic loci. Additionally, the close

linkage between genes in the MHC complex makes it difficult to assign disease associations to specific genes. Application of new sequencing methods may alleviate some of these issues and may yield important information for breeding for improved resistance to viral pathogens. None the less, it is clear that *BoLA* class I restricted cytotoxic T cell responses are primed by infection with bovine para-influenza virus 3 (BPIV3) (Bamford *et al*., 1995), BHV-1 (Hegde *et al*., 1999), FMDV (Guzman *et al*., 2008) and BRSV (Gaddum *et al*., 1996). Furthermore, CTL are important in clearance of BRSV from both the upper and lower respiratory tract of calves (Taylor *et al*., 1995). Thus it is not surprising that viruses of cattle and other species have also evolved complex mechanisms for evading the attention of CTL (Ambagala *et al*., 2005). For example, several viruses including FMDV, BPV and BVDV down regulate the expression of MHC class I on the surface of infected cells (Sanz-Parra *et al*., 1998; Araibi *et al*., 2004; Lee *et al*., 2009, respectively), and BHV-1 probably also affects expression of *BoLA* class I, as it inhibits one of the MHC class I peptide-processing enzymes, namely transporter associated with antigen processing (TAP) (Koppers-Lalic *et al*., 2003).

MHC class II

MHC class II molecules function primarily to present exogenous peptides to $CD4^+$ T helper (Th) cells. In cattle these consist of DR and DQ heterodimers, unlike humans who also have DP molecules (Lewin *et al*., 1999). There have been many studies investigating the associations between the polymorphisms present in class II genes and infectious disease (Lewin *et al*., 1999; Glass, 2004). *BoLA-DR* is a heterodimer of a monomorphic DRA chain and a highly polymorphic DRB3 chain. *BoLA-DRB3* is highly variable, with 104 different alleles reported so far (http://www.ebi.ac.uk/ipd/mhc/bola/) and there may be many more alleles. However, both the *DQA* and *DQB* genes are polymorphic and in some haplotypes they are duplicated. This can increase the potential range of peptides presented, as there is evidence for both inter- and intra-haplotype pairings of DQA and DQB to form functional molecules (Glass *et al*., 2000; Norimine and Brown, 2005). The suggestion that animals with duplicated haplotypes might have higher immune responses and therefore be more disease resistant in general (Glass *et al*., 2000) has had some support, as cattle with single DQ haplotypes are more susceptible to mastitis (Park *et al*., 2004). Induction of effective Th cells is essential for generating B cell and antibody responses. Cytokines produced by Th cells can also stimulate expansion of $CD8^+$ T cells and their activation into CTLs. Furthermore, $CD4^+$ T cells can acquire CTL activity, lysing cells expressing MHC class II molecules, such as antigen-presenting cells (APC). Therefore MHC class II processing and presentation pathways are involved in host defence against viral invasion. As with MHC class I, there is evidence that viral infection primes *BoLA* class II restricted $CD4^+$ Th cells in cattle, e.g. BRSV (Fogg *et al*., 2001), FMDV (Gerner *et al*., 2009) and BVDV (Collen *et al*., 2002). However, in cattle there is little direct evidence that *BoLA* class II polymorphism is associated with variability in protection against bovine viruses. Although a number of association studies have linked

polymorphisms in *BoLA-DRB3* with disease and/or vaccine outcome (Lewin *et al*., 1999; Glass, 2004), these have mainly been with non-viral pathogens, and clearly the same caveats apply as with the MHC class I gene associations (see above).

However, there is strong evidence to suggest that *BoLA DRB3* polymorphisms influence the outcome of BLV infection (see below), and our own evidence would suggest that *BoLA-DRB3* also influences the induction of protection against FMDV by putative peptide vaccines (Garcia-Briones *et al*., 2001). MHC restriction may play a particularly relevant role in protective responses to FMDV, because unlike other members of the picornaviridae family, FMDV has a prominent G-H peptide loop structure on its surface, which is highly antigenic and the main focus of virus-neutralizing antibodies, which are believed to form the major protective mechanism, although it is clear that other components of innate and acquired immunity, including T cell responses, are also essential (Collen, 1994; Summerfield *et al*., 2009). This region may also be immunodominant for T cell responses, and thus the binding affinity of loop region-derived peptides may determine the magnitude of the ensuing immune response (Glass *et al*., 1991). Our recent data suggest that *BoLA DRB3* class II polymorphisms, particularly those in the PBC, have a profound influence on the resultant antibody levels induced by a peptide containing this region (Baxter *et al*., 2009). These data, together with those of Garcia-Briones *et al*. (2001), suggest that *BoLA-DRB3* polymorphisms may play a relevant role in determining protection against FMDV. In particular, Baxter *et al*. (2009) found that polymorphisms in pocket 4 had the greatest impact on antibody response. Pocket 4 polymorphisms appear to be particularly relevant to variation in the immune response to many different antigens, both in cattle and in humans, possibly because it is located in the centre of the PBC, which makes it a critical anchor for peptides (Fu *et al*., 1995). Indeed, the strongest associations between *BoLA-DRB3* alleles and BLV resistance are also associated with polymorphisms in this anchor pocket (see below).

Although there is currently little evidence that bovine viruses use evasion strategies that target the MHC class II processing and presentation pathways, it would seem likely since there are many examples of strategies used by human viruses that inhibit presentation to $CD4^+$ T cells (Iannello *et al*., 2006), and indeed a recent paper by Lee and co-workers (2009) has shown that cytopathic BVDV infection in bovine monocytes results in lower levels of BoLA class II DR and DQ molecules. Thus *BoLA DR* and *DQ* genes and genes encoding their associated processing enzymes can also be considered as potential candidates for bovine genetic resistance to viruses.

Candidate gene summary

In general, the candidate gene approach for cattle has mainly identified candidates from the human or murine field and investigated identified polymorphisms that may or may not be important for host defence against viral infection.

Only a few studies have attempted to investigate their role in determining variation in resistance to viruses of importance for the cattle industry. However, the literature surveyed here would suggest that the candidate gene approach could have merit in the search for genetic factors that influence the outcome of viral infection in cattle.

Another way of identifying panels of candidate genes may be to investigate global gene expression changes following viral infection. Many studies have used this approach in humans and mice, but very few transcriptomic or proteomic studies have been conducted in cattle in relation to viral diseases, and none with the purpose of identifying genes involved in disease resistance. Three such studies on the host response to rotavirus, coronavirus and BVDV infection all reported that genes of the innate immune system were differentially regulated, including many of those described earlier (Aich *et al*., 2007; Shoemaker *et al*., 2009; Smirnova *et al*., 2009). The completion of the bovine genome sequence (The Bovine Genome Sequencing and Annotation Consortium, 2009) should also aid proteomic analysis of viral responses by cattle, as sequence information will improve the identity of candidate proteins. A recent study has demonstrated differential protein expression, particularly in immune-related proteins in macrophages infected with BVDV (Lee *et al*., 2009). Such studies, combined with SNP-based approaches, might result in more accurate predictions of candidate genes for resistance to viral infections of cattle.

Whole-genome Scans

Although the candidate gene approach may ultimately yield markers that are useful for breeding for resistance to viral infection, another approach that can be employed is to conduct whole-genome scans using markers across the entire genome. The identification of genotypes by this approach is covered elsewhere in this book, and will not be covered here. Suffice to say that the bovine genome has now been sequenced to 7.1 times coverage (The Bovine Genome Consortium *et al*., 2009) and a HapMap of bovine SNPs published (The Bovine HapMap Consortium, 2009). In the past, microsatellite markers have been used to conduct whole-genome scans, but although microsatellites are highly polymorphic, there are too few to reduce the size of the confidence interval of the chromosomal regions identified by quantitative trait loci analysis to a list of candidate genes amenable to further analysis. SNPs, on the other hand, are much more numerous, and the whole-genome approach may become a significant way of identifying causal genes. A further advantage of SNP-based studies is that the SNPs themselves may be sufficiently close to causal genes that they can be used directly to breed for disease resistance, even in unrelated populations. However, to the authors' knowledge, whole-genome scans for resistance to viral infections in cattle have not been reported in the literature, although a study is under way on bovine BRD (http://www.ars.usda.gov/is/pr/2008/080805.htm).

Host Phenotypic and Genetic Variation in Relation to Viral Infections in Cattle

In this section we provide examples of major viruses of cattle for which there is evidence of genetic variation in host response.

Bovine leukaemia virus (BLV)

BLV, the causative agent of bovine enzootic bovine leukosis, is an oncogenic exogenous C-type delta retrovirus which affects mainly cattle and to a lesser extent sheep, and has about 50% homology to human T cell leukaemia viruses type I and II, as well as to primate T cell tropic viruses. The virus targets B cells in both cattle and sheep. It is spread by transfer of infected cells in blood, milk, secretions, mainly through an iatrogenic route of contamination, and possibly also by blood-sucking insects or bats. In the EU it has almost been eradicated, but has a wide presence in other parts of the world, including the USA, where it is estimated that around 89% of dairy cattle herds are infected (Rhodes *et al*., 2003). Infected animals are persistently infected, but most cattle and sheep remain asymptomatic for several years before almost all sheep develop leukaemia with associated pathology. In contrast, about 30% of cattle develop a benign but persistent lymphocytosis (PL), with a small minority going on to develop fatal leukaemia. This pathogen may be responsible for considerable productivity losses even at the subclinical stage (Wu *et al*., 1989; Rhodes *et al*., 2003).

Although it is not entirely clear, it seems likely that immune-based surveillance keeps the virus in check until the viral load and/or mutations in oncogenes exceed a threshold beyond which the immune system can no longer contain the virus (for a recent review see Gillet *et al*., 2007). Inhibition of DNA repair enzymes by a viral protein (BLV-tax) may be the crucial step to transformation (Philpott and Buehring, 1999). Infected animals have detectable levels of antibodies, implying that infection of B cells may not have a detrimental impact on the humoral arm of the immune system. Recently Florins *et al*. (2008) have proposed that the host–pathogen interaction with BLV is subtly different in cattle and sheep and this could account for the marked differences in host outcome following infection. They suggest that ovine B cells infected with BLV have a higher proliferative rate compared with cattle, and this, combined with a higher death rate in infected ovine B cells compared with cattle, leads to enhanced cell turnover in sheep. They speculate that B cell turnover occurs in the spleen, with cytotoxic cells directed to the virus playing an important role. Such species differences may be a result of greater co-adaptation between cattle and BLV compared with sheep. Understanding the genetic basis for the above species differences could be very instructive in understanding the mechanisms of pathology and protection. In fact, sheep do not naturally transmit BLV, although it is not clear why. Many different breeds of cattle are susceptible to BLV infection (Xu *et al*., 1993; Udina *et al*., 2003; Konnai *et al*., 2006), and no breed differences in susceptibility have been reported, although dairy cattle are more commonly infected, and have a higher incidence of lymphosarcoma, probably because of management practices, not breed differences per se.

Evidence to date suggests that genetic factors may play a role at different stages of infection with BLV: from initial infection, to the likelihood of developing PL, through to the fatal lymphosarcoma. A heritability of 0.48 was reported for susceptibility to infection with BLV as measured by antibody (Burridge *et al*., 1979), albeit with a large standard error, due to relatively small numbers of animals in the study. In contrast, Detilleux and colleagues (1995) reported that in Holstein cattle, the heritability was close to zero for prevalence of anti-BLV antibodies, although selection for high predicted transmitting ability (PTA) for fat and protein had a significant effect on antibody level, suggesting that genetic factors are important at least for induction of antibody to BLV. However, the relationship between antibody titres and the likelihood of going on to develop clinical signs is not clear.

Early studies indicated that resistance to the development of the PL stage of the disease in cattle was associated with genes within the bovine *MHC* or *BoLA* and, since then, a wealth of studies have confirmed these findings in many different breeds (Xu *et al*., 1993; Lewin *et al*., 1999; Udina *et al*., 2003; Juliarena *et al*., 2008). The strongest links are to the classical class II gene, *DRB3*, and in particular a specific motif glu-arg (ER) at amino acid positions 70 and 71, which reside within pocket 4 of the DRB3 PBC that is associated with resistance to PL (Xu *et al*., 1993). Several different *DRB3* alleles have this motif, including *DRB3*0701*, **0703*, **0902*, **2101*, **2401*, **2703* and **2707* (plus another six alleles that have not been investigated in any MHC and BLV studies). As noted earlier, polymorphisms in pocket 4 appear to be particularly relevant to variation in the immune response. In a more recent study, Juliarena and co-workers (2008) found that the *DRB3*0902* allele has an odds ratio (OR) of greater than 8 for association with low pro-viral load, confirming an earlier smaller-scale study (Mirsky *et al*., 1998). In addition, Udina *et al*. (2003) found that *DRB3*0201*, which has a deletion at codon 65 changing the conformation of the peptide-binding pocket, was associated with resistance to PL development. Similar *DRB* associations have been found in sheep infected with BLV in that resistance to the development of lymphoma was associated with the RK motif in *OLA-DRB1* (*BoLA-DRB3* equivalent) genes of the ovine *MHC* (Nagoaka *et al*., 1999). It is probable that the RK motif gives a similar advantage to cattle (Udina *et al*., 2003), which would suggest that at least some of the aetiology of the disease in cattle and sheep must be similar. Several of the *BoLA*-related studies have found that the motif Val-Asp-Thr-Tyr (VDTY and/or VDTV) at amino acid positions 75–78 (position 78 is within pocket 4) confer susceptibility to BLV-induced leukaemia (Udina *et al*., 2003). There are three alleles (*BoLA DRB3*2701*, **2801* and **2802*) that have both the susceptible VDTY motif and the resistant E-R motif, but none of these have been detected in any experimental herds.

The majority of investigations into the role of genetics in BLV-associated morbidity and mortality have concentrated on the classical genes of the *BoLA* complex. However, there is some evidence that other genetic factors may play a role in susceptibility to PL in cattle (Wu *et al*., 1989), and resistance is also likely to be controlled by genes other than *DRB3* (Lewin *et al*., 1999; Kabeya *et al*., 2001). The *MHC* region is very gene dense and contains many

immune-related genes that may be in strong linkage disequilibrium with particular alleles of *BoLA class II DRB3*.

In terms of candidate genes, considerable evidence suggests that genetic factors influencing cell-mediated immunity (CMI) are likely to play an important role, as evinced by the strong associations with *DRB3* alleles that determine the strength of the T cell response to peptides presented by this molecule (Lewin *et al*., 1999). CMI-related responses during BLV infection involve both innate responses and acquired immune responses, with $CD8^+$ MHC class I restricted T cells, γδ T cells as well as $CD4^+$ class II restricted T cells (Kabeya *et al*., 2001). It is presumed that CMI suppresses BLV replication, but antibody responses probably also contribute to protection against infection (Kabeya *et al*., 2001). Pro-inflammatory cytokines such as TNFα, encoded within the *MHC*, are upregulated in macrophages and other innate immune cells in response to the early stages of infection, which can also lead to upregulation in T cells. TNFα plays a role in viral clearance, and TNFα expression has previously been reported to play a role in the pathogenesis of BLV, at least in sheep (Kabeya *et al*., 1999). A polymorphism (-824G) in the promoter region of TNFα is associated with lower transcription of TNFα in uninfected peripheral blood lymphocytes responding to a mitogen, as well as a higher pro-viral load and greater propensity to develop PL in infected cattle (Konnai *et al*., 2006). Differential expression of other cytokines involved in CMI including both Th1 type (interferon-γ (IFN-γ) and Th2 type (interleukin (IL)-4 and IL-10) cytokines appears to be aberrantly regulated at different stages of the disease (reviewed by Kabeya *et al*., 2001), with higher IFN-γ expression immediately following infection with BLV being associated with resistance to onset of PL, at least in sheep (Usui *et al*., 2007).

In addition, polymorphisms in alpha-lactalbumin have been reported to relate to the susceptibility of bovine lymphocytes to infection with BLV (Bojarojc-Nosowicz *et al*., 2008). The complexity of host immunity to BLV underscores the possibility of further genetic resistance and susceptibility loci being uncovered, although the strength of the MHC–DR association suggests that it should be possible to select for resistant alleles and/or remove animals expressing susceptibility alleles without influencing the overall diversity of the MHC region. Recently, Juliarena and colleagues (2009) demonstrated that antibody levels to several viral pathogens are not associated with the BLV-resistant allele, suggesting that selection based on this allele might not compromise resistance to other pathogens.

Bovine respiratory disease (BRD)

Respiratory infections of cattle are a major animal welfare problem and impose a considerable financial burden on the agricultural industry. Outbreaks of respiratory disease in calves have a complex aetiology and can be associated with a single pathogen or a combination of pathogens, including viruses (e.g. BRSV, BVDV, BPIV3, BHV-1), bacteria (e.g. *Mannheimia haemolytica*, *Pasteurella multocida*, *Haemophilus somnus*) and/or mycoplasmas (e.g. *Mycoplasma bovis*).

Analysis of genetic factors influencing BRD has shown that there are only small differences between different breeds of cattle for resistance to respiratory disease (Snowder *et al*., 2006). Braunvieh and Pinzgauer breeds appear to be most susceptible to respiratory disease (Muggli-Cockett *et al*., 1992; Snowder *et al*., 2006), and the incidence of disease was also high in Simmental and Limousin cattle. Furthermore, Herefords appeared to be more susceptible than composite breeds, and mortality associated with respiratory disease was greatest in Red Poll calves (Snowder *et al*., 2006). Heritability estimates for resistance to respiratory disease are low, ranging from 0.04 to 0.08 ± 0.1 (Muggli-Cockett *et al*., 1992; Snowder *et al*., 2006; Heringstad *et al*., 2007a), indicating that selection for resistance to respiratory disease would be slow, unless between-animal variability was very large. However, since calf respiratory disease has a complex aetiology, it is possible that selection for resistance to a defined pathogen may be more successful. In addition, estimates of heritability are generally based on all animals in a study, regardless of whether they have been challenged or not. In fact, heritability estimates increased in situations of increased incidence of BRD, suggesting that selection for BRD resistance would be greater if the phenotype for BRD resistance was known (Snowder *et al*., 2005).

BRSV is the single most important viral cause of lower respiratory tract disease in young calves (Stott and Taylor, 1985; Valarcher and Taylor, 2007). BRSV is closely related to HRSV, a major cause of bronchiolitis and pneumonia in infants and young children (Stott and Taylor, 1985), and 8 of the 11 proteins encoded by BRSV have over 80% homology with those of HRSV. However, the viruses are specific for their respective hosts and this appears to be dependent upon on a number of viral genes (Buchholz *et al*., 2000; Bossert and Conzelmann, 2002; Schlender *et al*., 2003). None the less, the epidemiology and pathogenesis of infection with these viruses are similar (Van der Poel *et al*., 1994). BRSV occurs in annual winter outbreaks and 70% of cattle have been infected by 9 months of age (Stott and Taylor, 1985; Valarcher and Taylor, 2007). BRSV replicates primarily in the superficial layer of the respiratory ciliated epithelium and type II pneumocytes (Viuff *et al*., 2002, 1996) and produces pathological changes including necrosis and proliferation of bronchial epithelial cells, peribronchiolar and perivascular infiltration by lymphocytes and monocytes and a bronchiolar exudate composed of neutrophils, desquamated epithelial cells and macrophages. Although both BRSV and HRSV are cytopathic in tissue culture, little or no cytopathic effects are seen following *in vitro* infection of differentiated, ciliated airway epithelial cell cultures (J.-F. Valarcher, R. Sadler, L. Hibbert, and G. Taylor, unpublished observations; Zhang *et al*., 2002). These observations suggest that the host response to virus infection plays a major role in RSV disease pathogenesis. RSV infection induces a rapid and pronounced innate inflammatory response and an adaptive response that may also play a role in disease pathogenesis (Collins and Graham, 2008). Therefore, genetic differences in the innate and/or adaptive immune response may determine the severity of disease. A role for genetic predisposition in severe RSV disease is indicated by differences in HRSV replication and pathology in inbred strains of mice (Prince *et al*., 1979;

Stark *et al*., 2002; Chavez-Bueno *et al*., 2005) and differences in the severity and frequency of HRSV bronchiolitis in different ethnic human populations (Holman *et al*., 2004; DeVincenzo *et al*., 2005). In addition, a study of over 12,000 twin pairs showed that there was increased concordance of severe RSV disease in identical twins over fraternal twins (Thomsen *et al*., 2008). These findings suggest that genetic factors accounted for about 20% of the individual susceptibility to develop severe RSV infection and support the suggestion that genetic factors may contribute to differences in the susceptibility of individual calves to BRSV. As noted earlier, a study is under way to explore variation across the genome in cattle in relation to BRD, and it was recently reported, in a meeting abstract, that an SNP on BTA20 may be related to the incidence of BRD as well as to a fat-related trait (Garcia *et al*., 2008).

Genetic susceptibility to severe HRSV infection in young children has been investigated using candidate gene approaches. The findings from these studies are summarized in Table 6.3 and indicate that severe HRSV infection in young children is associated with polymorphisms mainly in genes involved in innate immunity. Polymorphisms in genes encoding cytokines and chemokines, genes encoding proteins involved in pathogen recognition or intracellular signalling, and genes encoding surfactant proteins have also been associated with severe HRSV bronchiolitis. However, there is a lack of reproducibility for many of the genetic associations, which may be related to factors such as study design and sample size.

A study that analysed 384 SNPs in 220 candidate genes in 470 children hospitalized for RSV bronchiolitis identified a total of 22 SNPs in 21 genes that had a significant association with severe RSV disease (Janssen *et al*., 2007). SNPs in the vitamin D receptor (*VDR*), *JUN*, *NOS2A* and *IFNA5* genes had the strongest association at both the genotype and allele level with severe RSV disease, highlighting the importance of innate immunity in susceptibility to HRSV bronchiolitis. The role of *VDR* in HRSV disease is unclear. However, *VDR* has been implicated in downregulating IL-12 and IFN-γ production, and these cytokines are important in promoting CMI and recovery from virus infections. *JUN* is part of transcription factor AP-1, which is a mediator of pro-inflammatory cytokine production. Nitric oxide derived from inducible nitric oxide synthetase (iNOS) is also a pro-inflammatory mediator (Ricciardolo *et al*., 2004). The significance of the association between severe HRSV disease and SNPs in IFNA5, IFNA13 and STAT1, which were associated only at the allele level, is difficult to interpret as both HRSV and BRSV are poor inducers of IFN-α/-β and are resistant to the antiviral effects of IFN-α/-β (Schlender *et al*., 2000; Valarcher *et al*., 2003; Spann *et al*., 2005). However, HRSV infection in IFN-non-responsive mice (STAT1 knockout) resulted in a Th2-biased response and increased pulmonary pathology compared with wild-type animals, despite similar levels of virus replication (Durbin *et al*., 2002), suggesting that innate IFNs contribute to the inflammatory response.

Like other pathogens, RSV is recognized by innate immune receptors such as TLRs, leading to activation of NF-κB and induction of chemokines and other pro-inflammatory mediators. There is evidence that the HRSV F protein binds to and activates TLR4, which is the primary signalling receptor for lipopolysaccharide

Table 6.3. Studies on genetic associations with severe human respiratory syncytial virus (HRSV) disease.

Gene	Polymorphism	Sample size	Association	Reference
SP-A	9 SNPs in SPA1 and SPA2	86	Coding polymorphisms in SPA1 and SPA2 associated with severe RSV disease	Lofgren *et al*. (2002)
SP-B	-32 G/T, T1311I, 5781 A/C, L176F, R272H	131	SP-B haplotypes associated with severe RSV disease	Puthothu *et al*. (2007a)
SP-C	N138T, N186S	131	SP-C haplotypes associated with severe RSV disease	Puthothu *et al*. (2006b)
SP-D	M11T, A160T, S270T	84	M11T associated with severe RSV disease	Lahti *et al*. (2002)
MBL	3 SNPs which affect levels of serum MBL	55	No association	Kristensen *et al*. (2004)
TLR4	D299G, T399I	99	SNPs found more frequently in severe RSV compared with mild disease	Tal *et al*. (2004)
	D299G	236	No association	Paulus *et al*. (2007)
	D299G, T399I	54	SNPs not detected in the Japanese population	Inoue *et al*. (2007)
	D299G, T399I	105	Associated with symptomatic RSV disease in infants at high risk of severe disease	Awomoyi *et al*. (2007)
	D259G and T359I	131	D259G and haplotypes associated with severe RSV disease	Puthothu *et al*. (2006a)
TLR8	2007 C/G	470	2007G allele associated with severity of disease	Janssen *et al*. (2007)
CD14	-159 C/T	99	No association	Tal *et al*. (2004)
	-159 C/T	131	No association	Puthothu *et al*. (2006a)
	-159 C/T, -550 C/T	54	-550 C allele associated with severe RSV disease in Japanese children	Inoue *et al*. (2007)
IFN-γ	874 A/T	77	Association between high-producer IFN-γ genotype -874 T) and more severe RSV disease	Gentile *et al*. (2003)
	rs2069718, rs2430561, rs2069705	156	No association	Mailaparambil *et al*. (2008)

Continued

Table 6.3. Continued.

Gene	Polymorphism	Sample size	Association	Reference
IFN-γR1	rs2234711, rs1327474	156	No association	Mailaparambil *et al*. (2008)
IFN-α5	rs10757212 (453 C/T)	156	No association	Mailaparambil *et al*. (2008)
	rs10757212 (453 C/T)	470	Associated with severe RSV disease at both the allele and genotype level	Janssen *et al*. (2007)
IFNA13	-603 C/T	470	-603T allele associated with disease severity	Janssen *et al*. (2007)
IFN-αR1	rs2257167, rs2254315	156	No association	Mailaparambil *et al*. (2008)
ISG15	rs15842, RS1921	156	No association	Mailaparambil *et al*. (2008)
IFI27	rs3814821, rs7141881	156	No association	Mailaparambil *et al*. (2008)
IFI44	rs11577395	156	No association	Mailaparambil *et al*. (2008)
VDR	T1M	470	Associated with severe RSV disease at both the allele and genotype level	Janssen *et al*. (2007)
JUN	750 G/A	470	Associated with severe RSV disease at both the allele and genotype level	Janssen *et al*. (2007)
NOS2A	2757 G/A	470	Associated with severe RSV disease at both the allele and genotype level	Janssen *et al*. (2007)
TNFα	-308 G/A	207	No association	Hoebee *et al*. (2004)
	-1211 C/T, -1037 C/T, -488 A/G	151	TNFα haplotypes associated with severe RSV disease	Puthothu *et al*. (2009)
	-308 G/A	77	No association	Gentile *et al*. (2003)
	-857 C/T	470	Association at the genotype level with severity of disease	Janssen *et al*. (2007)
TGF-β1	10 C/T, 25 C/G	77	Low TGF-β producer genotypes associated with low O_2 saturation at admission to hospital due to RSV	Gentile *et al*. (2003)
IL-4	-1098 T/G, -589 T/C, -144 C/T, -33 T/C, G8375A, A8412C	105	Association between -589T, -33T, 8375G and 8412A alleles & severe RSV disease	Choi *et al*. (2002)
	-590 C/T	207	Association between -590T and severe RSV disease	Hoebee *et al*. (2004)

Continued

Table 6.3. Continued.

Gene	Polymorphism	Sample size	Association	Reference
	-589 C/T	131	Associated with severe RSV disease only in the context of IL-13 polymorphisms	Puthothu *et al.* (2006c)
IL-4R	I50V, Q551R	207	No overall association	Hoebee *et al.* (2004)
	E400A	470	Associated with severe RSV disease	Janssen *et al.* (2007)
	-3223 C/T	470	-3223 C/C associated with severe RSV disease	Janssen *et al.* (2007)
IL-5	-746 T/C	105	No association	Choi *et al.* (2002)
IL-6	-174 G/C	77	Low-producer IL-6 genotype (-174C/C) associated with more severe RSV disease	Gentile *et al.* (2003)
IL-8	8 SNPs in IL-8 gene and 55 in the flanking regions	580	Association between -251 T/A and severity of RSV disease	Hull *et al.* (2000, 2004)
	-251 A/T, -781 C/T	131	No association	Puthothu *et al.* (2006d)
IL-8RA	M32R, S276T, R335C	131	No association	Puthothu *et al.* (2006d)
IL-9	-345 A/G	207	No association	Hoebee *et al.* (2004)
IL-10	-1082 G/A, -819 T/C, -592 A/C	77	Low-producer IL-10 genotypes (-1082 A) associated with RSV pneumonia	Gentile *et al.* (2003)
	-592A/C	207	No association	Hoebee *et al.* (2004)
	5876 C/T, 4949 T/C, 1547 C/T, 919 G/T, 627 C/A, 854 C/T, 1117 A/G, 3585 T/A	580	-1117G and -3585A associated with infants requiring mechanical ventilation	Wilson *et al.* (2005)
	-592 A/C	470	Association at the genotype level with severity of disease	Janssen *et al.* (2007)
IL-13	-1512 A/C, -1112 C/T, G2044A	105	No association	Choi *et al.* (2002)
	-1512 A/C, -1112 C/T, G2044A	131	-1112T allele associated with severe RSV disease	Puthothu *et al.* (2006c)
IL-15	667 C/T	470	667T allele associated with severe disease	Janssen *et al.* (2007)
IL-17	627 G/A	470	Association at the genotype level with severity of disease	Janssen *et al.* (2007)

Continued

Table 6.3. Continued.

Gene	Polymorphism	Sample size	Association	Reference
IL-18	-607 A/C, -137G/C, 113 T/G, 127 C/T, 5304 A/G, 133 G/C	154	Severe RSV disease associated with 133 G/C and with all 6 SNPs by haplotype analysis	Puthothu *et al.* (2007b)
RANTES	-28 C/G, -403 G/A, In.1.1 T/C	106	-28 C/C, -403 G/A and In. 1.1 T/T combined genotype associated with severe RSV disease	Amanatidou *et al.* (2008)
CCR5	-1835, -2459, -2554, -2733, Δ32	580	-2459G and -2554T associated with severe RSV disease	Hull *et al.* (2003)
CX3CR1	V249I, T280M	82	Association between 280 M/M or T/M and severe RSV disease	Amanatidou *et al.* (2006)
ICAM-1	K469E, 20788 A/G	154	No association	Krueger *et al.* (2006)
VCAM-1	-833 C/T	154	No association	Krueger *et al.* (2006)
	-1594 T/C	470	-1594C allele associated with severe RSV disease	Janssen *et al.* (2007)
E-selectin	S128R, H468Y	154	No association	Krueger *et al.* (2006)
CD28	407+309 A/G	470	G allele associated with severe RSV disease	Janssen *et al.* (2007)
HLA	HLA A, HLA B		No association	Isaacs *et al.* (1989)
IGHG	IGHG3(b) and (g), IGHG1(f) and (a), IGHG2 (n) and (-n)	49	IHGH2 (-n/-n) and IGHG(bf-n) alleles associated with severe RSV disease	Aurivillius *et al.* (2005)

(LPS), and it has been suggested that this interaction contributes to the pathogenesis of RSV infection (Kurt-Jones *et al.*, 2000; Haeberle *et al.*, 2002). Several studies have shown that polymorphisms in *TLR4* (Asp299Gly and Thr399Ile), which are associated with hypo-responsiveness to inhaled LPS and increased susceptibility to Gram-negative bacterial infections (Arbour *et al.*, 2000; Lorenz *et al.*, 2002), are associated with severe HRSV bronchiolitis in infants (Tal *et al.*, 2004; Puthothu *et al.*, 2006a; Awomoyi *et al.*, 2007). However, the findings were not consistent (Janssen *et al.*, 2007; Paulus *et al.*, 2007), and other studies in mice demonstrated that RSV replication and host responses were similar in *TLR4*-deficient and wild-type mice (Ehl *et al.*, 2004). Thus, the role of TLR4 in the pathogenesis of RSV infections is not clear. The observation that BRSV induces a TLR4-dependent NF-κB response in cell culture (Lizundia *et al.*, 2008), together with the findings that bovine *TLR4* is

highly polymorphic with a number of SNPs within or close to the putative ligand-binding domain (White *et al*., 2003) – SNPs that affect TLR4 expression (Sharma *et al*., 2008) and SNPs that are associated with increased susceptibility of cattle to *Mycobacterium avium* subsp. *paratuberculosis* (Mucha *et al*., 2009) – suggests that studies to determine a genetic association between severe BRSV disease and SNPs in bovine *TLR4* may be worth exploring. Although there is evidence that HRSV activates other TLRs, e.g. TLR3 (Rudd *et al*., 2006) and TLR2 (Murawski *et al*., 2009), a genetic association between SNPs in these *TLR*s and severe HRSV bronchiolitis in man has not been found (Janssen *et al*., 2007). However, an association between HRSV disease severity and an SNP in TLR8, which is activated by ssRNA, was found in male infants (Janssen *et al*., 2007).

Several studies have identified an association between polymorphisms in surfactant protein genes and susceptibility to HRSV (Table 6.3). Surfactant proteins are involved in opsonization and phagocytosis of a variety of bacterial and viral pathogens. HRSV F and G proteins interact with surfactant protein A (SP-A), promoting uptake by macrophages and inhibiting infection of cells (Ghildyal *et al*., 1999; Barr *et al*., 2000). Furthermore, SP-A deficient mice have more severe HRSV infection than their wild-type littermates (LeVine *et al*., 1999). Similarly, HRSV infection is more severe in SP-D-deficient than in wild-type mice (LeVine *et al*., 2004). These observations demonstrate that surfactant proteins play an important role in pulmonary defence against HRSV.

The neutrophil is the predominant cell type in the airways of calves with BRSV pneumonia and infants with HRSV bronchiolitis. IL-8 (CXCL8), which is a potent neutrophil attractant, is present in high levels in airway secretions of infants with HRSV (Smyth *et al*., 2002), and IL-8 mRNA is increased in pneumonic tissue from gnotobiotic calves infected with BRSV (L. Hibbert, J.-F. Valarcher and G. Taylor, unpublished observations). Infants with polymorphisms associated with increased secretion of IL-8 were likely to exhibit more severe HRSV disease (Hull *et al*., 2000, 2004). Other inflammatory mediators, such as IL-6, TNF-α, RANTES (CCL5), MIP-1α (CCL3) and MCP-1 (CCL2), or their mRNAs, are also increased in respiratory secretions from children hospitalized with HRSV (McNamara *et al*., 2005) and in pneumonic lung tissue from gnotobiotic calves infected with BRSV (L. Hibbert, J.-F. Valarcher and G. Taylor, unpublished observations). Of these chemokines, an association between polymorphisms in TNF-α and RANTES and severe HRSV bronchiolitis has been detected in some but not all studies (see Table 6.3). However, two polymorphisms in *CCR5*, which encodes the receptor for RANTES and MIP-1α, are associated with severe HRSV bronchiolitis (Hull *et al*., 2003). Although the role of the promoter SNP of *CCR5* is not clear, it has been suggested that it may increase expression of CCR5. A polymorphism in another chemokine receptor, *CX3CR*, has also been associated with increased risk for severe HRSV bronchiolitis in a study involving a small number of individuals (Amanatidou *et al*., 2006; Bukreyev *et al*., 2006). The HRSV G protein contains a CX3C motif, which has limited sequence homology with fractalkine (CX3CL), and appears to bind to CX3CR and have chemoattractant

activity *in vitro* (Tripp *et al*., 2001). There is evidence from studies in mice that the HRSV G protein can act as a fractalkine agonist and reduces the recruitment of cytotoxic T cell leukocyte (CTL) and other leukocytes to the lungs (Harcourt *et al*., 2006). However, other studies suggest that the CX3C region of the HRSV G protein enhances the pulmonary CTL response to RSV (Bukreyev *et al*., 2006). Although the G protein of the majority of BRSV strains also contain a CX3C motif (Furze *et al*., 1997; Langedijk *et al*., 1997), some field isolates of BRSV have been identified in which one or several of the cysteines have been replaced with either an alanine or an asparagine (Spilki *et al*., 2006; Valarcher *et al*., 2000). Therefore, the role of SNPs in *CX3CR* in susceptibility to RS viruses is not clear.

HRSV infection of lung epithelial and endothelial cells upregulates adhesion molecules such as *IACM-1* and *VCAM-1* (Wang *et al*., 2000; Arnold and Konig, 2005), which direct leukocytes to the site of infection, and enhances the adhesion of bacteria to epithelial cells (Avadhanula *et al*., 2006). In addition, ICAM-1 may play a role in uptake of HRSV by epithelial cells (Behera *et al*., 2001). Although studies found no evidence that SNPs in *ICAM-1*, *VCAM-1* and *E-selectin* genes predisposed to severe HRSV bronchiolitis (Krueger *et al*., 2006), an allele in the *VCAM-1* gene appeared to be protective (Janssen *et al*., 2007).

Studies in mice indicate that the balance of the T cell response is important in determining the outcome of HRSV infection (Openshaw and Tregoning, 2005). $CD8^+$ T cells play an important role in the clearance of HRSV in mice (Graham *et al*., 1991) and of BRSV in calves (Taylor *et al*., 1995) and produce IFN-γ, which promotes a protective Th1 response. However, both exuberant Th1- and Th2-biased responses have been associated with severe HRSV disease in the mouse (Openshaw and Tregoning, 2005). These observations have prompted analyses of polymorphisms in Th1 and Th2 cytokine genes in infants with severe HRSV infections and have identified a genetic association at the 5q31 cytokine cluster with increased risk of severe HRSV disease (Forton *et al*., 2009). This locus contains a cluster of Th2 cytokine genes, *IL-4*, *IL-13* and *IL-5*, and the study demonstrated that an IL-4/IL-13 haplotype, which is associated with increased IL-13 production, confers an increased risk of severe HRSV bronchiolitis in early infancy. Associations between severe HRSV disease in infants and *IL-4*, *IL-13* or *IL-4R* have also been reported by other groups (see Table 6.3). The significance of these observations for BRSV in calves is unclear. Although IL-4 mRNA has been detected in pneumonic lungs and thoracic duct lymph of BRSV-infected calves, but not in uninfected animals (McInnes *et al*., 1998; Gershwin *et al*., 2000), IL-13 mRNA was not detected (L. Hibbert, J.-F. Valarcher and G. Taylor, unpublished observations). However, the numbers of BRSV-infected calves studied were small. In contrast to the failure to detect IL-13 mRNA, levels of IL-15 and IFN-γ mRNA were increased in pneumonic lungs from BRSV-infected gnotobiotic calves (G. Taylor, unpublished observations). High producer IFN-γ genotype and an IL-15 allele have both been associated with severe HRSV bronchiolitis in infants (Gentile *et al*., 2003; Janssen *et al*., 2007). Polymorphisms in IL-10, which downregulates cell-mediated immune responses, have also been associated with increased risk of severe HRSV bronchiolitis in

some studies, but not in others (see Table 6.3). Children homozygous for the IL-10 promoter SNP -592 C or A have a higher risk of severe RSV disease than do heterozygous carriers (Janssen *et al*., 2007).

An SNP associated with altered FcεR1 expression levels and allergic disease in man is associated with severe HRSV bronchiolitis in infants at both the allele and genotype level (Janssen *et al*., 2007). BRSV-specific IgE is produced following experimental infection of calves with BRSV and the level of antibody correlates with severity of clinical disease (Gershwin *et al*., 2000). These observations suggest that studies to determine a genetic association between severe BRSV disease and SNPs in FcεR1 may be worth exploring. *IGHG2*(-n) alleles and *IGHG2*(-n/-n) genotypes, which are associated with low specific IgG2 antibody responses to bacterial antigens such as pneumococci and *Haemophilis influenzae* type b polysaccharide in man, are also associated with an increased risk of severe HRSV bronchiolitis in infants (Aurivillius *et al*., 2005). The BRSV-specific antibody response in calves to a commercial vaccine is also under genetic control (O'Neill *et al*., 2006). However, an association between low BRSV-specific antibody responses and risk of severe BRSV disease has not been explored.

The studies summarized above demonstrate that genetic susceptibility to HRSV bronchiolitis is a complex trait. However, genes involved in innate immunity show the strongest association with HRSV bronchiolitis. Since the pathogenesis of BRSV infection in calves shares many similarities with that of HRSV in infants, it is likely that genes associated with innate immunity in cattle will also contribute to the severity of BRSV respiratory disease.

Bovine herpesvirus 1 (BHV-1)

BHV-1 is a member of the subfamily *Alphaherpesvirinae* of the family *Herpesviridae*, and causes infectious bovine rhinotracheitis and infectious pustular vulvovaginitis. Southern blot analysis of 16 type I IFN genes from 98 unrelated, mixed-breed cattle inoculated intranasally with BHV-1 identified alleles at three *IFN* loci (*IFNB1, IFNW4* and *IFNW8*) that were significantly associated with severe clinical signs of disease (Ryan *et al*., 1993). A second allele at the *IFNB1* locus was associated with milder disease. The role of type I IFN in susceptibility to BHV-1 infection and severity of disease is not clear. BHV-1 is only moderately sensitive to the antiviral effects of bovine IFN-α (Hohle *et al*., 2005). However, stimulation of innate immune responses, including IFN-α, by CpG oligodeoxynucleotide in newborn lambs reduced shedding of BHV-1 (Nichani *et al*., 2006).

Foot-and-mouth disease virus (FMDV)

FMDV is endemic in large parts of the world, though not in the UK, the EU, the USA or Australia. Many countries where FMDV is endemic routinely vaccinate using inactivated viral vaccines. In contrast, in non-endemic regions the policy is to quarantine and slaughter. The causative agent of the disease is an apthovirus

member of the *Picornaviridae* family. It has a single-stranded RNA genome which is non-enveloped and is made up of a capsid with icosahedral symmetry (Grubman and Baxt, 2004). The symmetry is made up from 60 copies of four proteins, Vp1–Vp4. There are seven distinct serotypes of the virus: O, A, C, SAT 1–3 and Asia 1, and the RNA nature of FMDV makes it highly mutable (Domingo *et al*., 2005), adding to the difficulty of designing appropriate control measures. FMDV is highly contagious for cloven-hoofed animals, and because it is extremely stable, persists in the environment. It can also be carried and potentially transmitted by other non-infectable species such as horses and humans. Infection of cattle generally occurs via the respiratory route, with infected animals exhaling the virus in aerosol form (Grubman and Baxt, 2004). Once the clinical signs of fever, blisters and lameness are present, viral presence is obvious (Grubman and Baxt, 2004). However, animals are highly infectious before they develop these signs, at which point it is too late to quarantine individual animals. The highly infectious nature, high mutability and broad species infectivity of FMDV, together with animal welfare and economic considerations (due to culling, trade restrictions and other indirect effects, e.g. on tourism), make FMDV the single most important livestock disease and have prompted calls for the progressive global control of this disease (Rweyemamu *et al*., 2008).

Since the 2001 UK outbreak, which cost an estimated US$13 billion (Grubman and Baxt, 2004), research around the world has mostly been directed to developing safer, more efficacious vaccines (Saiz *et al*., 2002). Despite the rapidity with which FMDV spreads, there is some evidence in the literature that genetic resistance to FMDV may exist in cattle. There is a report of a Charolais cow that did not develop FMD despite being in contact with clinically affected animals (cited in Morris, 2007). Furthermore, six descendents of the cow were resistant or developed mild symptoms during a second outbreak 14 years later. A later report also suggests that genetic factors may play a role in the response to vaccination with inactivated viral vaccines (Samina *et al*., 1998) but, in general, such considerations have not been evaluated and the significance of these observations is unclear. However, following FMDV infection, a proportion of ruminants can carry the virus for long periods in their pharyngeal tissue (Salt *et al*., 1996). The role of the host innate or adaptive immune response in the development of the carrier state is not known, but may be influenced by host genetics. Certainly the response to natural infection, as well as the response to vaccination in cattle, is highly variable, opening up the possibility that unexplained genetic factors may play an important role. As discussed earlier, MHC class I and MHC class II genes may influence the humoral and cellular response to FMDV, and we have also found evidence that other chromosomal loci affect the level and class of antibody to a peptide derived from FMDV (R. Leach and E.J. Glass, in publication).

There are considerable species differences in the course and transmission of this virus, from pigs that produce the highest viral titres, to sheep and goats that are often subclinical or asymptomatic (Alexandersen and Mowat, 2005). Some species, such as Old World camels, appear to be resistant to natural infection (Wernery *et al*., 2006). Although rarely fatal in cattle, the degree of morbidity approaches 100%, with calves being particularly susceptible.

The molecular basis for the species' variability in resistance and susceptibility is unclear, and further investigation is warranted, as this might suggest new methods for control. Interestingly, although adult mice are generally considered non-permissive to FMDV infection, certain strains of mice have proved highly susceptible (Salguero *et al*., 2005), again suggesting genetic factors may be important in determining relative resistance and susceptibility to FMDV. Whether or not mouse studies would be useful in identifying genetic components controlling response to FMDV in livestock remains untested. Indeed, it is not clear whether genetic resistance could be usefully harnessed as part of a wider control package, by breeding for resistance. However, understanding the underlying basis could suggest new control strategies.

Rinderpest virus (RPV)

RPV is a morbillivirus belonging to the family *Paramyxoviridae*, and is responsible for a contagious viral infection of cloven-hoofed animals characterized by profuse diarrhoea and inflammation and erosion of various mucous membranes. The mortality rate in highly susceptible animals can exceed 90%. However, the severity of RPV infection varies considerably between different breeds of cattle. Susceptible animals such as unhumped cattle (*Bos taurus*), water buffalo (*Bubalus bubalus*) and yaks (*Bos grunniens*) develop severe acute clinical disease. In contrast, clinical disease is less severe in humped (zebu) cattle (*Bos indicus*) (Wohlsein and Salik, 2006) and in Grey Steppe cattle, which were reported to shed RPV for months, in the absence of clinical signs (Pastoret *et al*., 2006). The mechanisms responsible for these differences in susceptibility are not known. One possible component of the innate response to virus infection that may contribute to differences in susceptibility of *B. taurus* and *B. indicus* cattle is *Mx1*. *Mx1* is an antiviral protein that is induced by IFN-α/-β. A phylogenetic tree of the 11 genotypes of bovine *Mx1* suggests that the genotypes observed in Brahman cattle, which belong to *B. indicus*, were a substantial genetic distance from the genotypes observed in *B. taurus* (Nakatsu *et al*., 2004). Although human MxA protein has antiviral activity against measles virus, which is also a morbillivirus (Schnorr *et al*., 1993), there is no information on the relative effects of *B. indicus* and *B. taurus Mx1* on replication of RPV. Furthermore, other paramyxoviruses appear to be resistant to the antiviral effects of bovine *Mx1* (LeRoy *et al*., 2005).

Malignant catarrhal fever virus (MCFV)

MCF is a serious, often fatal, lymphoproliferative disease of cattle, bison, deer and other ungulates. MCF is caused by several viruses in the genus *Rhadinovirus* of the family *Herpesviridae*, subfamily *Gammaherpesvirinae*. The MCF subgroup of viruses contains at least ten members, five of which are known to cause disease. Ovine herpesvirus 2 (OvHV-2), which is endemic in most sheep, is the major cause of MCF worldwide. Alcelaphine herpesvirus 1 (AlHV-1),

which is endemic in wildebeest, causes the wildebeest-associated form of MCF in cattle and other species. Bison (*Bison bison*) and Bali cattle (*Bos javanicus*) are highly susceptible to MCF. Nevertheless, approximately 20% of commercial American bison become infected with OvHV-2 without developing clinical disease. Analysis of MHC class IIa polymorphisms in 77 bison with clinical MCF and 112 bison that were infected without clinical disease revealed a statistically significant association between resistance to MCF and *BoLA/Bibi-DRB3* alleles (Traul *et al*., 2007). Thus, *DRB3*0801* was significantly associated with resistance to MCF and *DRB3*0602* was significantly associated with clinical disease. However, the role of the MHC class II molecules in determining susceptibility to MCF disease is not known. A number of studies have demonstrated that MHC class I-restricted CTLs are required to control acute gammaherpesvirus infections (Stevenson and Efstathiou, 2005), and it is likely that such lymphocytes play a role in controlling OvHV-2 infection in bison. In the light of these studies, there may also be an association between MHC class I haplotypes and resistance to OvHV-2.

Lumpy skin disease (LSDV)

LSDV causes a range of symptoms in cattle from subclinical to acute. Skin lesions can cover the entire body and infection can occasionally be fatal (Babiuk *et al*., 2008). Certain breeds of cattle are more susceptible to LSDV than others, especially those that are thin-skinned such as Jersey and Guernsey breeds. The morbidity rate varies from 3% to 85%, suggesting that a variety of factors may influence clinical disease. Poxviruses employ a variety of immune evasion strategies such as the production of homologues of cytokines, chemokines and their receptors as well as non-homologous chemokine-binding proteins (Alcami, 2003). These virus immune-evasion strategies highlight the importance of chemokines and cytokines in antiviral defence and suggest that polymorphisms in host genes encoding cytokines, chemokines and/or their receptors may contribute to differences in clinical disease produced by poxvirus infections.

Conclusions and Future Perspectives

It is clear that many obstacles and difficulties still lie ahead if we are to achieve any goals of breeding for resistance to viral infection in cattle. We need to consider further how best to identify relevant disease resistance genes. Clearly genotyping is entering a new era with the advent of cheaper sequencing strategies and newly available SNP panels, and in the near future it will become routine to consider sequencing whole genomes. Considerably more genetic and genomic data will become available in the very near future, necessitating greater computing power and more sophisticated bioinformatics to sift out what is relevant and what is not. We need more databases that bring related information together in easily accessible formats such as the multi-species QTL database set up by Reecy and colleagues (AnimalQTLdb; http://www.animalgenome.org/QTLdb/).

The era of comparative genomics is now upon us, with data and genome sequences for many different species, and will be increasingly exploited in the future to further understand the interaction between hosts and viruses, and has the potential to lead to new insights into what determines the host range for viruses, and what factors lead to changes in host susceptibility. The ability to compare different genomes, QTL regions and gene sequences will also impact on the choice of candidate genes to investigate. We have undertaken such an approach to identify TLRs and downstream pathway components as 'hot' candidates for disease resistance traits (Jann *et al*., 2009).

The number of candidate genes that may play a role in resistance has also grown, along with a much greater understanding of the host–viral dialogue, especially in the early stages following viral entry. It is clear that the host has evolved many mechanisms for sensing and destroying viral invaders, and although this in turn has resulted in counter-strategies by viruses, it seems likely that natural genetic variation in many of the host components of the innate immune system has evolved in turn to control the onslaught of viral pathogens.

The ability to identify SNPs in candidate genes in relevant breeds should also be improved by the recent publication of the HapMap for cattle. The accompanying publically available databases of SNPs (The Bovine HapMap Consortium, 2009) should allow a broader, more systematic search for polymorphisms in candidate genes. None the less, the lists are likely to be extensive as indicated by Table 6.3, which catalogues associations between candidate gene SNPs and RSV infections in humans. However, even when potential SNPs are identified that are non-synonymous and in probable functional domains, very few studies in cattle have been conducted on their actual functional consequences, not even in *in vitro* experiments.

The phenotype gap, however, remains a major issue, with few appropriate data being collected. In some cases it may be more sensible to simply consider identifying animals that remain productive in specific environments, in the face of unknown clinical and subclinical infections, and instead of measuring specific responses to infection, use performance traits as the readout phenotype, as exemplified by the studies of Clapperton *et al*. (2008). However, this approach may not identify animals that are resistant to specific pathogens under different environmental conditions, and may well not be a very efficient process, as growth and feed efficiency characteristics are also determined by factors unrelated to disease resistance. Indeed, Snowder *et al*. (2007) reported that there was no apparent genetic correlation between respiratory disease and growth and meat quality traits in beef cattle. Furthermore, it may well be that indigenous breeds could harbour useful disease resistance genes, which, if they were identified using appropriate SNP markers, could be used to breed in the desirable characteristics without the accompanying breed-related low productivity traits. Isolated populations may also have accumulated different polymorphisms in different genes to sustain viability against the same disease, and therefore crosses of breeds might bring together two distinct genetic strategies to overcome a specific pathogen, resulting in greater resistance than in the individual breeds themselves (additive effects). Conversely, the increased rate of

inbreeding as a consequence of livestock breeding programmes aimed at improving economically important traits may result in a loss of diversity in some cattle populations, which could make them less able to deal with rapidly evolving or new pathogens. For example, as mentioned previously, polymorphism in the MHC molecules increases the range of peptides that can be recognized by T cells, and thus increases the likelihood that a heterozygous individual will be able to cope with any particular pathogen. Although heritabilities for disease resistance traits often appear low, it is still possible to select for resistance as has been shown for mastitis (Heringstad *et al*., 2007b), suggesting that breeding for viral resistance may be a viable scenario.

The overwhelming evidence suggests that harnessing genetics could provide new approaches to disease control for the rapidly changing environment that cattle farmers are facing, both in developed and developing countries.

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7 Viral Diseases in Pigs

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Summary

Genomic approaches have expanded our understanding of genes and gene pathways and quantitative trait loci (QTL) controlling traits of economic importance in pig production, recently including health traits and disease resistance. Efforts are under way to use novel tools including pig gene arrays, single nucleotide polymorphisms (SNPs) chips, genome-wide association studies (GWAS) and advanced bioinformatics to find new candidate genes and biological pathways associated with host resistance, viral disease processes and mechanisms, and biomarkers that account for control of responses to viral pathogens and vaccine efficacy in targeted pig populations. This chapter focuses on the advances made on using genomic approaches to define swine resistance to viral pathogens, particularly for the most economically important viruses, porcine reproductive and respiratory syndrome virus (PRRSV) and porcine circovirus (PCV). These studies will have substantial impact for the pig industry: it is now possible to include the use of biomarkers for basic health traits alongside a broader set of markers utilized for selection of pigs for improved performance and reproductive traits, as well as for pork quality.

Introduction

Traditional genetic approaches have been effectively used by the swine industry to enhance feed efficiency, meat production, leanness and reproductive traits, yet limited work has addressed swine health (Mellencamp *et al*., 2008; Green, 2009). The future impact of animal genomics in animal health research was reviewed in a 2007 meeting and a roadmap for future research developed (Archibald *et al*., 2008; Pinard-van der Laan and Gay, 2008). A critical goal is the development of genomic-based assays to select for disease susceptibility traits in targeted animal populations and defined animal production systems.

 Breeding for Disease Resistance in Farm Animals, 3rd Edition (eds S.C. Bishop *et al.*)

Advances in Swine Genetics

Swine genome researchers have worked effectively with industry and used genetic approaches to identify quantitative trait loci (QTL), and specific genes and genetic variants, that control feed efficiency, meat production, pork leanness and reproductive traits (Rothschild *et al*., 2007; Rothschild and Plastow, 2008). The swine genome sequence is almost (~98%) completed (www.sanger.ac.uk/Projects/S_scrofa/; Humphray *et al*., 2007). Molecular markers, particularly microsatellites (MS) and single nucleotide polymorphisms (SNPs), along with pig SNP chips and methods to identify copy number variants, are now available for high-throughput screening for QTL (Karlskov-Mortensen *et al*., 2007; Fadista *et al*., 2008; Ramos *et al*., 2009; Vingborg *et al*., 2009).

In the last decade swine genome research has expanded from identifying genes associated with production and reproduction traits to swine health, well-being and disease resistance traits (Lunney, 2007; Archibald *et al*., 2008; Mellencamp *et al*., 2008; Edfors-Lilja and Torremorell, 2009). Early work mapped QTL for immune capacity in the pig (Edfors-Lilja *et al*., 1994, 1998); this has been expanded recently (Clapperton *et al*., 2005, 2007, 2009; Wattrang *et al*., 2005; Wimmers *et al*., 2008, 2009; Reiner *et al*., 2009a,b,c) to identify QTL associated with more detailed phenotypic traits. Studies by Galina-Pantoja *et al*. (2006) found that the proportion of several peripheral cell subsets appeared to predict growth during the entire productive life of the pig. Wilkie and Mallard's studies focused on selecting pigs for high immune response (Magnusson *et al*., 1997, 1998; Raymond and Wilkie, 1998; Wilkie and Mallard, 1999) and concluded that genetic variation in response to certain antigens and to *Mycoplasma hyorhinis* exists. Although pathways and mechanisms involved in resistance were not characterized, it was concluded that the genetic variation was polygenic, regulating both innate resistance and acquired immunity.

Broader pig-mapping studies, using SNP chips and microarrays for gene expression, can now determine which genes or genomic regions may correlate with improved pig health, vaccine responses or resistance to specific infectious agents (Tuggle *et al*., 2007; Phatsara *et al*., 2008; Ponsuksili *et al*., 2008; Chen *et al*., 2009; Steibel *et al*., 2009). With the advances in animal genomics, genetic studies have expanded from mapping individual QTL and identifying candidate genes to GWAS, which in turn can lead to genome-wide selection (GWS). Results from these studies should provide producers with sets of DNA markers, molecular pathways and protein biomarkers to identify pigs that require fewer therapeutic treatments and exhibit better disease resistance against specific pathogens.

Major Swine Viral Infections

To address the topic of this chapter it is important first to identify the major swine viral infections for which researchers would address genetic resistance. Table 7.1 lists the most important swine viruses and identifies the areas of the pig industry internationally for which each virus has its greatest impact. First on

Table 7.1. Major swine viral infections.

Virus	Genetic resistance	Commercial importance[a]	Vaccine availability	Trans-boundary importance[b]	Zoonotic importance	Selected background reference(s)
Porcine reproductive and respiratory syndrome virus (PRRSV)	Yes	+++++	++ for homologous but limited for heterologous virus	Yes for variants associated with porcine high fever disease (PHFD)	None known	Neumann *et al.* (2005); Cho and Dee (2006); Tian *et al.* (2007)
Porcine circovirus (PCV); post-weaning multi-systemic wasting syndrome (PMWS)	Yes	++++	Effective vaccines	No	None known	Segalés *et al.* (2005); Opriessnig *et al.* (2007a); Finsterbusch and Mankertz (2009); Gillespie *et al.* (2009)
Swine influenza virus (SIV)	Yes	+++	Effective vaccines; must be updated to new reassortants	No	High, due to new viral reassortants	Vincent I.E. *et al.* (2008)
Pseudorabies virus; Aujesky's disease (PRV)	Yes	Eradicated in numerous countries	Effective vaccines	No	None known	Bouma (2005)
Coronaviruses (CoV) (transmissible gastroenteritis virus (TGEV); porcine respiratory coronavirus (PRCV))	Unknown	++++ TGEV ++ PRCV	Effective vaccines	Yes TGEV No PRCV	None known	Saif (1996, 2004); Enjuanes *et al.* (2005)
Hepatitis E	Unknown	+	Unknown	No	Likely	Meng *et al.* (1998)

Continued

Table 7.1. Continued.

Virus	Genetic resistance	Commercial importance[a]	Vaccine availability	Trans-boundary importance[b]	Zoonotic importance	Selected background reference(s)
Porcine endogenous retrovirus (PERV)	Unknown	Endogenous	Unknown	No	Recombination potential – xenotrans-plants	Gorbovitskaia *et al.* (2003); Magre *et al.* (2003); Wilson (2008)
Classical swine fever (CSF)	Yes	++++	++	+++	No	Paton and Greiser-Wilke (2003)
Foot and mouth disease (FMD)	Unknown	++++	+++ but serotype specific	+++++	No impact for veterinary species	Kitching *et al.* (2005)
Vesicular stomatitis (VS)	Unknown	+++	++	+++	None known	Fernández *et al.* (2008); Wilson *et al.* (2009)
African swine fever (ASF)	Yes	++++	None	+++	No	Costard *et al.* (2009)
Nipah virus	Unknown	++++	None	Under investigation	Under investigation	Chua *et al.* (2000); Tee *et al.* (2009)
Ebola virus	Unknown	++++	None	Under investigation	Under investigation	Barrette *et al.* (2009)

[a]Impact of disease for large commercial operations. [b]Trans-boundary diseases are those defined as notifiable by the World Organisation for Animal Health (OIE, http://www.oie.int).

the list is porcine reproductive and respiratory syndrome virus (PRRSV), which is a threat to pig production worldwide (Neumann *et al*., 2005). Periodically, severe PRRSV outbreaks result in abortion 'storms' accompanied by high sow mortality (Lowe *et al*., 2005; Cho and Dee, 2006). The virus is transmitted congenitally and via semen of infected boars (Christopher-Hennings *et al*., 2001, 2008). There are type 1 European and type 2 North American PRRS viruses that share only about 67% nucleotide sequence identity (Wensvoort *et al*., 1991; Benfield *et al*., 1992). Nursing and growing pigs have chronically recurring illness, pneumonia, delayed growth and potentially high mortality.

The international risks associated with PRRS were exacerbated in 2006 by reports of 'pig high fever disease (PHFD)', a highly pathogenic pig disease in China for which type 2 PRRSV has been identified as the single most prominent virus (Tian *et al*., 2007), although data indicate that interactions among respiratory pathogens may be responsible. Because of the PHFD threat, the World Organisation for Animal Health (OIE) has classified certain PRRSV variants as notifiable trans-boundary diseases (http://www.oie.int). Unfortunately, because PRRSV is a ribonucleic acid (RNA) virus, its genome changes over time and heterologous strains quickly arise as major threats to the swine industry. As a result, current commercial vaccines are only partially effective against heterologous virus (Mateu and Diaz, 2008; Kimman *et al*., 2009). Characterization of genetically encoded host resistance mechanisms could lead to identification of genes that help pigs control PRRSV or that prevent or decrease losses associated with PRRS (Lewis *et al*., 2007; Lunney, 2007).

Another virus with a worldwide distribution, porcine circovirus type 2 (PCV2), has long been thought to be responsible for economical loss associated with the post-weaning multi-systemic wasting syndrome (PMWS) that was most prominent in Europe (Segalés *et al*., 2005; Madec *et al*., 2008). The emergence in recent years of a more highly pathogenic strain, PCV2b, caused major losses in the industry with classic porcine respiratory disease complex (PRDC) in neonates and early finisher pigs. The PCV2-associated disease (PCVAD) syndrome spread quickly through North American swine herds (Opriessnig *et al*., 2007b; Ramamoorthy and Meng, 2008). The dynamics of PCV2 spread and development of PMWS is a major concern (Dupont *et al*., 2009). Luckily PCV vaccines are effective, cross-protective and now readily available for PCV2 control. However, the role of genetics in vaccine efficacy and in pig resistance to PCV2 infection has had limited study (Opriessnig *et al*., 2006, 2009).

The last decade has seen worldwide concern about the potential for avian influenza virus to become zoonotic in pigs and humans (Thacker and Janke, 2008). This threat has been exacerbated by the recent appearance of 'swine flu' or A/H1N1 in 2009 (Cohen, 2009). Efforts for pig influenza control have been aimed at determining the many emerging subtypes of swine influenza virus (SIV) infection and developing appropriate vaccines to prevent infection with the latest reassorted virus (Vincent *et al*., 2008). The potential of zoonotic infections (i.e. transmission between pigs and humans) and expression of novel reassorted viruses further aggravates the worldwide threat to both the pig industry and human health. Paul *et al*. (2003) reviewed the potential zoonotic threat of certain exogenous viruses, highlighting their characteristics, pathogenesis in

swine and detection methods, as well as their potential xenozoonotic risk for organ transplantation.

Pseudorabies virus (PRV) and coronaviruses (CoV) (transmissible gastroenteritis virus (TGEV) and porcine respiratory coronavirus (PRCV)) have continued to cause disease in pigs, although successful biosecurity and vaccines have resulted in disease prevention and control in developed countries. For PRV, numerous countries have effectively used differentiation of infected from vaccinated animals (DIVA) vaccine-based programmes to eradicate the virus in commercial pig populations (Stegeman, 1995). The emergence of severe acute respiratory syndrome (SARS) in China in the early 2000s affirmed what veterinary virologists had previously recognized, i.e. that CoVs can cause fatal respiratory or enteric disease in animals with increased threats due to interspecies transmission and wildlife reservoirs (Saif, 2004). Improvement of CoV vaccines has been an important result of the global response to SARS (Enjuanes, *et al*., 2005).

The OIE has established a list of notifiable diseases that cause substantial animal losses and are highly infectious (http://www.oie.int). For swine the notifiable trans-boundary or foreign animal diseases caused by viruses include foot-and-mouth disease (FMD), African swine fever (ASF), classical swine fever (CSF), swine vesicular disease (SVD) and vesicular stomatitis (VS). Sporadic outbreaks of VS result in economic losses because VS is a reportable disease that clinically mimics FMD (Fernández *et al*., 2008; Wilson *et al*., 2009). An outbreak of one of the OIE notifiable viral diseases results in immediate restrictions on worldwide sales of a country's pork products. Thus, because of the financial impact on a country's economy, major efforts are made to prevent these diseases in unaffected countries; for affected countries, efforts are aimed at improving control and prevention of these diseases through biosecurity and vaccines to control their spread (Domenech *et al*., 2006; Belák, 2007).

Definition of Disease Resistance

Research on genetic resistance to viral pathogens of pigs aims to identify and understand the allelic variation associated with resistance/susceptibility to numerous factors including pathogen infection, replication, tissue tropism, transmission, persistence and speed and regulation of innate and adaptive immune responses. Thus, it is important at first to state how one defines viral disease resistance.

As obligate intracellular pathogens, viruses typically must first bind to and enter the host cell, since they require the host machinery to replicate. Thus, complete, or sterilizing, resistance would be expected in pigs that did not express the virus receptor, similar to the *Escherichia coli* F4ab/ac receptor in pigs (Edfors-Lilja and Torremorell, 2009). Future work is expected to reveal (and likely even to lead to the design of) alterations in cellular receptors that prevent, or alter the efficiency of, viral attachment or entry. However, for certain viruses there is more than just one receptor, e.g. for PRRSV it is clear that two receptors are involved, namely sialoadhesin (SIGLEC1) and CD163. Specifically,

CD163 correlates with productive infection; sialoadhesin is required for internalization whereas CD163 is essential for viral entry, probably during uncoating (Calvert *et al*., 2007; Van Gorp *et al*., 2008; Patton *et al*., 2009). In addition, many viral receptors are probable essential cell surface proteins and thus deletion would not result in viable pigs.

For many viral diseases, disease resistance is the result of a combination of factors including viral dose at infection, local tissue replication, environmental factors, concomitant infections and speed and intensity of innate and adaptive immune responses. Clinical signs result from these interactions as do infection-associated growth effects (Nguyen *et al*., 2006). However, clinical disease traits such as respiratory scores and rectal temperatures may be poor indicators of virus levels, pathological damage or growth (Doeschl-Wilson *et al*., 2009). Moreover, pigs may appear to be resistant to viral infection, but actually replicate the virus and really be tolerant to viral pathology and be sources of viral shedding for the remainder of the herd. Indeed viral tolerance is a complex trait probably involving many genes, including those involved in growth and immune responses. Moreover, age, previous infection and vaccination status, nutritional plane, stress, reproductive status and external environment can alter the interactions between the pig host and viral pathogen (Batista *et al*., 2004). Finally, some, none, or all of these factors may be involved in determining viral persistence, another major risk factor for certain viruses. Persistence impacts new entrants to the herd, providing a source of continuing viral infection.

Overall, identifying genes or pathways that determine viral disease resistance/susceptibility is a complex process (Bishop and MacKenzie, 2003). Breeding for disease resistance is a balancing act (Stear *et al*., 2001; Gibson and Bishop, 2005; Mellencamp *et al*., 2008). In the past, breeders may have inadvertently created selection indices that include traits with undesirable effects, e.g. viral susceptibility. In the future, genomics will hold the promise of providing powerful DNA markers for desired production traits as well as markers to minimize disease effects.

Resistance to Specific Viral Infections

Genetic resistance to PRRSV infection

Breed differences clearly play a role in determining resistance/susceptibility of pigs to PRRSV. Halbur *et al*. (1998) found that Duroc pigs when infected with PRRSV had greater serum ELISA sample-to-positive (S/P) ratios, lower average daily gain and increased severity of PRRSV-induced lesions in the lung than Meishan pigs. A.L. Vincent *et al*. (2005, 2006) compared pigs from two commercial pig lines to a challenge with PRRSV at 6 weeks of age and found that macrophage responses are partially predictive of breed and line associations with PRRSV resistance. They used an *in vitro* fluorescence-activated cell sorting (FACS) assay to determine the percentage of PRRSV-infected macrophages. A line derived from Large White pigs with a high percentage of PRRSV-infected macrophages was compared with a line

derived from Duroc and Pietrain pigs with a low percentage. The latter line had more severe clinical disease 10 days post-infection (dpi), although differences between lines diminished by 21 dpi (Vincent *et al*., 2006). Ait-Ali *et al*. (2007) showed significantly reduced viral replication in pulmonary alveolar macrophages (PAMs) from Landrace pigs compared with other breeds tested. They suggested that factors intrinsic to the Landrace breed, including abundance and localization of the PRRSV co-receptor, sialoadhesin, may be responsible for this reduced or delayed response to PRRSV. Reduced or delayed growth of PRRSV was temporally associated with high levels of tumour necrosis factor and interleukin-8 (IL-8) mRNA, suggesting cytokines as key contributors to the innate immune response to PRRSV infection. With a sow model, Lowe *et al*. (2005) found that genetics may affect the rate of PRRSV-induced abortions. Data indicated that clinical protection was possibly due to levels of circulating interferon-γ (IFN-γ)-secreting cells in three of four farms studied. However, there was considerable variation between farms and between sows within farms. The authors noted that increasing cell-mediated immunity, as evidenced by IFN-γ-secreting cells, within infected herds has the potential to decrease clinical reproductive disease.

Petry *et al*. (2005), using a nursery pig 14-day PRRSV-infection model, showed that disease symptoms were more severe for pigs from lines selected for lean growth at the acute phase of the selection. Mean phenotypic responses indicated that lean growth-selected Hampshire Duroc (HD) pigs were more susceptible to PRRSV than pigs selected for improved reproductive traits. Infected HD pigs gained less weight from 0 to 14 dpi; had greater rectal temperatures and greater viraemia in serum, lung and bronchial lymph nodes (BLN); and had greater incidence of lung lesions. Follow-up studies showed that, based on principal-component analyses, pigs could be assigned to high- and low-viral burden groups. Low-PRRSV burden pigs had high weight gain, low viraemia and few lung lesions (Petry *et al*., 2007). After infection, low expression of IFN-γ in cDNA and in serum was correlated with PRRSV resistance. Importantly, high pre-infection serum levels of IL-8 were significantly associated with PRRSV-resistant, low-viral burden pigs (Petry *et al*., 2007). Combined with follow-up gene expression array studies by Bates *et al*. (2008), these studies indicated that important genetic associations could be revealed for fine-mapping of candidate genes for PRRSV resistance and determining the causative alleles.

Studies are now under way in the USA, conducted by the PRRS Host Genetics Consortium (PHGC), using a similar nursery pig model (42 days of infection), to assess pig resistance/susceptibility to primary PRRSV infection and viral persistence. Cross-bred pigs from high-health farms, donated by commercial sources, are infected with PRRSV. Results from each of the first six trials of 200 pigs have confirmed that all pigs become PRRSV-infected, yet growth is not highly correlated with PRRSV burden (Lunney, 2007; Lunney *et al*., 2010b). Multivariate analyses of viral load and weight data have identified PHGC pigs in different virus/weight categories; studies are planned to assess serum cytokine and whole-blood gene expression studies

comparing data from PRRSV-resistant/maximal growth pigs with PRRSV-susceptible/reduced growth pigs and PRRSV-susceptible/maximal growth pigs. Genomic DNA of all pigs will be genotyped with the Porcine SNP60 SNPchip and GWAS started. All data have been entered into the secure PHGC relational database. Overall, the PHGC project should enable researchers to verify important genotypes and phenotypes that predict resistance/susceptibility to PRRSV infection.

Doeschl-Wilson *et al*. (2009) reported significant line differences in growth during infection. The line that exhibited faster growth rate in the absence of infection suffered more severe clinical disease and greater reduction in weight growth after infection compared with the other line. They reported weak correlations between growth and disease-related traits as well as most clinical and pathological traits. Indeed, the line with the advantage during the acute period had greater virus levels at the later time point, indicating that the interaction between host and virus is quite complex. Doeschl-Wilson *et al*. (2009) concluded that the growth–susceptibility relationship may be determined by at least three possible underlying mechanisms that are under partial genetic control: (i) PRRSV infection leads to reduction in food intake with direct consequences on growth; (ii) immune response has a direct effect on growth through common genetic pathways other than those controlling food intake; and (iii) growth and immune response compete for the same resources, leading to a trade-off between growth and immunity.

A consistent result of many studies is that pigs from lines or breeds with high levels of reproduction (Meishan, Large White and NE Index line) are more resistant to the effects of the virus than pigs from lines selected for lean growth rate (Duroc, Pietrain and HD) (Halbur *et al*., 1998; Petry *et al*., 2005, 2007; A.L. Vincent *et al*., 2005, 2006). This finding could be coincidental, but may be related to the stage of growth at which responses to virus were measured, which was in the growing pig. Because PRRSV is specific to the respiratory tract, infection of the lungs may have greater negative effects on pigs with greater rates of lean growth. However, in an extensive study of reproductive performance data on 8098 litters from 1820 sows from a commercial farm with Landrace, Large White, Pietrain, Meishan, Duroc composite and various crosses, Lewis *et al*. (2009a) found that Meishan-line sows showed greater susceptibility to PRRSV than the sows from major European lines studied. They compared performance differences between baseline and disease phase for sows of different parities and lines. PRRSV caused significant production losses, and for reproductive traits impacts were greater in early parities, e.g. number of mummified piglets was greater at lower parities (1–5) than in older sows (parities 6–11). Line differences and interactions were also detected, highlighting a greater impact of PRRSV on the Meishan line than on their European counterparts. Lewis *et al*. (2009b) compared methods for assessing disease impact: a date/threshold method based on veterinary diagnosis and a threshold/threshold method based on trends in underlying performance data. The latter resulted in a data set with the threshold/threshold method that was

slightly smaller (1977 litters from 1526 sows) than that from the date/threshold method (3164 litters and 1662 sows) but that revealed even more pronounced impacts of PRRS on performance. Specifically, genetic variation in piglet losses during PRRSV outbreaks is independent of genetic variation in the same traits in healthy herds. The authors also concluded that parity affected host genetic resistance to PRRSV and should be considered when designing management strategies.

Responses controlling resistance to PRRSV infection

From a genetic selection standpoint, it would be desirable to select on a trait in uninfected pigs that is correlated with a response in infected pigs. A.L. Vincent *et al*. (2005) found that macrophage responses are only partially predictive of breed and line associations with PRRSV resistance. Petry *et al*. (2007) tested levels of serum immune proteins, the serum cytokines, and determined that high pre-infection serum levels of IL-8 were significantly associated with PRRS virus-resistant, low-viral burden pigs. After infection, low expression of IFN-γ in cDNA and in serum was also correlated with PRRSV resistance. Thus they predicted that genetic associations would be important for fine-mapping candidate genes for PRRSV resistance and thereby determining the causative alleles. Exactly which immune response endows full protection against homologous or heterologous PRRSV infection, however, is still an enigma. Lunney *et al*. (2010a) affirmed that concomitant changes in serum immune protein expression, IL-8, IL-1β and IFN-γ, are linked to PRRSV clearance. Studies of the same pigs affirmed that selected SLA class I and II genes are correlated with reduced PRRSV burden and with protective immune responses, cytokine levels and serum neutralizing antibody levels (Lunney *et al*., unpublished data).

Infection with PRRSV affects expression of many immune genes. Using real-time PCR analyses, Petry *et al*. (2007) reported that infection with PRRSV increased expression of 11 innate and T helper 1 immune markers in RNA produced from lung or BLN at 14 dpi. Gene expression was evaluated in a 2 × 2 × 2 factorial design. There was significant upregulation in lung, lymph or both of infected pigs relative to controls for all but one gene, but importantly, significant downregulation for certain immune genes in the low-viral burden pigs, relative to littermate controls. Ait-Ali *et al*. (2008) analysed porcine PAMs gene expression that implicated a dynamic differential regulation of the type-I IFN and chemokine transcripts during the first hours of infection with entry of the virus into macrophages, helping to define the mechanism underlying PRRSV replication in susceptible cells.

Over the last 5 years the capacity to analyse gene and protein expression in swine has been greatly expanded (see Tuggle *et al*., 2007 for a review). Swine functional genomic studies have been advanced by the availability of swine microarrays: NRSP8-Qiagen 12,500 long oligo probe arrays developed in 2003 (Zhao *et al*., 2005, 2006); Affymetrix arrays 20,000

probes in 2004 (Wang *et al*., 2007, 2008); and the 20,200 Illumina long oligo probe 'Pigoligoarray' in 2006 (Steibel *et al*., 2009). This set of functional genomic tools provides a platform for all researchers to evaluate local tissue responses to infectious agents that modulate immune function and induce unique gene expression patterns. Ait-Ali *et al*. (2008) showed that USP18 transcript accumulation is associated with susceptibility of PAM to PRRSV. However, there is a delay in upregulation of the porcine USP18 transcripts when compared with the classical polyI:C-induced type I IFN response transcript accumulation. This mimics the delayed type I IFN signalling seen early during infection in monkey MARC-145 cells and porcine PAMs (Wang *et al*., 2001; Miller and Fox, 2004; Miller *et al*., 2008). Overexpression of porcine USP18 in MARC-145 cells leads to reduced replication and/or growth of PRRS (Ait-Ali *et al*., 2009).

Genini *et al*. (2008) profiled *in vitro* Lelystad-PRRSV-strain virus replication in PAMs using Affymetrix microarrays and real-time PCR. Of the 1409 differentially expressed transcripts identified by analysis of variance, 2, 5, 25, 16 and 100 differed from controls by a minimum of 1.5-fold at 1, 3, 6, 9 and 12 h post-infection (hpi), respectively. A PRRSV infection effect was detectable as early as 3–6 hpi, and was characterized by a consistent downregulation of gene expression, followed by the start of the host innate immune response at 9 hpi. As reported by others, the expression of IFN-α was not upregulated, although IFN-β was upregulated. Moreover, few IFN-inducible, or common antiviral, genes were found to be differentially expressed. They reported a predominance of anti-apoptotic transcripts, e.g. IL-10, and a shift towards a T-helper cell type 2 (not the IFN-γ-associated T-helper 1) responses within 12 hpi, affirming that PRRSV had developed sophisticated mechanisms to escape host defences.

Bates *et al*. (2008) used NRSP8-Qiagen arrays to assess gene expression in RNA extracted from lung and BLN tissue of the seven highest- and seven lowest-PRRSV burden pigs from the Petry *et al*. (2005, 2007) studies, using a control reference design. Line and treatment effects were significant for 38 and 541 oligos, respectively, in both lung and BLN. Treatment–class interactions existed for expression of CCAAT/enhancer-binding protein, NF-κBIα, thioredoxin-interacting protein, major facilitator superfamily domain containing 1 (MFSD1) and other genes. These studies highlight possible genetic associations for fine-mapping PRRSV resistance-associated candidate genes.

In the future there will be even more complex studies using the newer arrays, sophisticated bioinformatic pathway analyses and more samples from local tissues as well as whole blood collected from pigs after PRRSV infection or vaccination. Proteomic analyses of macrophage responses to PRRSV infection have revealed function alterations induced by PRRSV (Zhang *et al*., 2009). Future studies should provide more details on the genes, proteins, pathways and networks that respond *in vivo* and *in vitro* to PRRSV infection and vaccination. They should help to reveal novel control mechanisms and alternative target genes and pathway functions that encode PRRSV resistance and endow protective antiviral responses.

Resistance to PCV2 infection and PMWS

Whether or not genetics and/or breed of the host has any influence on susceptibility or resistance to PMWS has also been discussed, based on field observations among pig breeders and veterinarians, specifically with regard to boar lines. Lopez-Soria *et al*. (2004) demonstrated a dramatic difference in post-weaning mortality due to PMWS in piglets derived from boars of a Large White × Duroc cross (up to 26.3%) compared with piglets of boars from a Large White × Pietrain cross (up to 5.9%) and piglets derived from pure-bred Pietrain boars (up to 2.1%). However, Rose *et al*. (2005) produced data that rejected the protective effect of the Pietrain breed. Another study demonstrated a predisposition to PCV2-induced disease and lesions in Landrace pigs compared with Duroc and Large White (Opriessnig *et al*., 2006). Together, these results suggest that the genetic make-up of the pig does play an important role during PCV2 infection and PMWS development, enabling some pigs to fight and overcome the infection while others suffer an unfavourable disease course.

Opriessnig *et al*. (2009) compared 39 Landrace and 39 Pietrain pig responses to PCV2 infection under experimental conditions with 13 control and 26 infected pigs for each breed, after waning of passively acquired anti-PCV2 antibodies. Onset of seroconversion and concentrations of anti-PCV2-IgM, anti-PCV2-IgG and anti-PCV2 neutralizing antibodies were similar in infected Landrace and Pietrain groups, as was the amount of viral DNA and plasma cytokine concentrations. The severity of PCV2-associated microscopic lesions was different: Landrace-PCV2 pigs had significantly more severe lymphoid lesions than the Pietrain-PCV2 pigs. Interestingly, passively acquired anti-PCV2-antibodies waned more quickly in Pietrain pigs (by ~12 weeks) than in Landrace pigs (~18 weeks of age and beyond). Thus a genetic difference exists between these two breeds of pigs in susceptibility to PCV2-associated lesions, as well as in persistence of protective maternal antibodies.

Resistance to pseudorabies virus (PRV)

As early as 1984, Rothschild and his colleagues analysed humoral immune responses to PRV vaccines (Rothschild *et al*., 1984). In a study of 518 pigs they found that vaccination efficacy varied between breeds, noting that vaccine trials should not neglect this potential source of variation. Later work on PRV and atrophic rhinitis vaccines (*Bordetella bronchiseptica* bacterin) used data from 988 pigs from 119 litters farrowed in a three-breed diallel cross-breeding experiment (Meeker *et al*., 1987a,b). Serum antibody response levels to both vaccines were not associated with backfat thickness or growth traits. Heritability for anti-PRV titre was 0.18 ± 0.09, with variability also attributable to maternal effects. The estimated heritability indicated that, if improved immune response to vaccines is desired, selection may be useful.

Pseudorabies was one of the first examples of using genetics to dissect the underlying regions of the genome associated with resistance to a porcine viral disease. Reiner *et al*. (2002) mapped QTL for differences in resistance/susceptibility to PRV infection between European Large White and Chinese

Meishan pigs, based on a genome-wide scan (85 MS markers) of 89 F2 pigs challenged intranasally at 12 weeks of age. All pigs developed clinical signs, i.e. fever, from 3 to 7 dpi. Further, the pure-bred Large White pigs, the F1 and three-quarters of the F2 animals, but none of the Meishan pigs, developed neurological symptoms and died or were euthanized. QTL for appearance/non-appearance of neurological signs were found on chromosomes 9, 5, 6 and 13 and explained 10.6–17.9% of F2 phenotypic variance. Significant QTL effects for rectal temperature after PRV challenge were found on chromosomes 9, 10 and 11. The authors noted that the QTL are in proximity to important candidate genes that are assumed to play crucial roles in host defence against PRV.

The availability of swine leukocyte antigen (SLA) and PRV viral gene arrays, as well as swine long oligo arrays, has enabled simultaneous analysis of viral and host gene expression (Flori *et al*., 2008a,b). These authors showed that expression of several genes involved in the SLA class I antigenic presentation pathway (SLA-Ia, *TAP1*, *TAP2*, *PSMB8* and *PSMB9*) was downregulated with PRV infection of swine PK15 epithelial cells, thus contributing to viral immune escape from class I immune pathways. These studies also identified differential expression of genes involved in the immune response, the apoptosis pathway, nucleic acid metabolism and cytoskeleton regulation during viral infection. This combined host/virus expression approach should help to decipher viral host response evasion strategies.

Resistance to notifiable trans-boundary diseases

Would genetically resistant pigs be an option for a notifiable trans-boundary disease? Because of the highly infectious nature of the OIE list A diseases, only pigs that were completely resistant to infection would probably be considered in countries that are free of the disease. Thus researchers would have to identify such pigs and prove full resistance (not inapparent carrier status) to various isolates of the virus. In contrast to countries free of notifiable trans-boundary disease, the use of genetically resistant animals can be a major benefit for eradication efforts in countries where these diseases are endemic. They could provide healthy stock while reducing disease incidence and further spread.

Warthogs and bush pigs are ASF virus (ASFV)-resistant, yet become infected and have minimal lesions (Oura *et al*., 1998). In numerous countries ASF-resistant pigs have been identified, these being pigs that survived an outbreak in an endemic country (Penrith *et al*., 2004), yet funds to support needed follow-up resistance studies have not been forthcoming, and genetic resources have been potentially lost. For CSF, Depner *et al*. (1997) reported breed-related differences. Blacksell *et al*. (2006) noted the delayed incubation time of native Moo Laat pigs compared with improved Large White × Landrace cross-bred pigs, although both suffered 50% mortalities at 18 and 11 dpi. Indeed the authors commented that this apparent resistance (actually disease delay) may have contributed to the maintenance and spread of CSF disease, since contact (and thus disease spread) is normally low.

Based on results from notifiable trans-boundary disease control efforts in other countries, US authorities have considered a range of options for their control.

These could include ring vaccination for prevention of disease spread. Vaccination with modified live virus strains is effective in preventing losses in countries where CSF is enzootic, but is unlikely, on its own, to eliminate infection entirely. In countries that are free of disease, or where eradication is in progress, vaccination is normally prohibited for trade reasons. Thus, identification of pigs with genetic resistance to notifiable trans-boundary diseases could be an important alternative.

Candidate Genes and Swine Health

As noted in the discussion above, numerous immune proteins have been identified as important factors in viral disease resistance. As studies progress with each viral infection, SNPs for specific candidate genes, such as for the ILs and IFNs, are being probed for their effects on viral resistance. Sang *et al*. (2008, 2009) have investigated the role of host-defence peptides and Toll-like receptors (TLRs) as innate antiviral effectors in PRRSV infection. Shinkai *et al*. (2006) identified SNPs in many porcine TLR genes; Uenishi and Shinkai (2009) have predicted their role in determining vaccine design and breeding for disease resistance. The MX1 locus has been identified with differential susceptibility to SIV (Nakajima *et al*., 2007; Palm *et al*., 2007).

For pig health, inheritance of specific alleles within the swine major histocompatibility complex (MHC; termed SLA) positively influences disease and vaccine responses (Lunney *et al*., 2009). The MHC is one of the most gene-dense regions in the swine genome, consisting of three major gene clusters, the SLA class I, class III and class II regions, that span ~1.1, 0.7 and 0.5 Mb, respectively, making the swine MHC the smallest among mammalian MHCs so far examined. The Immuno Polymorphism Database-MHC (IPD-MHC) website (http://www.ebi.ac.uk/ipd/mhc/sla/) serves as the repository of all SLA-recognized genes and their allelic sequences (Ho *et al*., 2009a). The recent development of molecular typing techniques for SLA class I and II alleles (Ho *et al*., 2009b, 2010) has opened up the possibilities of identifying specific alleles controlling antiviral responses in pigs. Luetkemeier *et al*. (2009) noted that, based on the genomic structure of SLA class II region, no skewing of SLA diversity has occurred despite regional differences in selective breeding and environments of the European- and Asian-origin pig DNAs analysed.

There is clear evidence that SLA antigens are modulated during viral disease responses, e.g. to ASFV, an indication of the role of these molecules in controlling infectious diseases (Gonzalez-Juarrero *et al*., 1992a,b). Viral interactions with dendritic cells (DCs) have important consequences for immune-defence function. The expression of MHC II and CD80/86 on the surface of DCs infected with PCV2 was not modulated; nor did PCV2 induce DC maturation, yet virus persisted within myeloid DCs in the absence of virus replication (I.E. Vincent *et al*., 2005). SLA alleles are intimately associated with identity of viral T cell epitopes such as the FMDV synthetic pentadecapeptides that stimulate class-II restricted T helper cell proliferation; and IFN-γ enzyme-linked immunospot assays (ELISPOTs) were identified and shown to represent class II- and class I-restricted helper and cytolytic T cell epitopes (Gerner *et al*., 2006).

Even though no common FMDV epitope was found, there was one overlapping peptide that may prove useful for the design of novel vaccines against FMDV. Preliminary studies with PRRSV have indicated that SLA class I and II alleles regulate PRRSV levels and important antiviral immune responses (Lunney *et al*., in preparation). Other implications of SLA alleles and gene expression on antiviral responses are reviewed in Lunney *et al*. (2009).

Conclusions

Since the early 2000s, we have seen major progress in swine immunology and genetics, particularly in understanding the role of genetics in normal immunity and in controlling infectious disease and vaccine responses. As reviewed in this chapter, major advances have been made in the identification of genes and alleles that influence pig viral replication, persistence and clinical disease. Knowledge and annotation of the complete swine genome sequence will enhance future studies. New immunological reagents, coupled with molecular and bioinformatic tools, have facilitated identification of biological pathways associated with host resistance, disease processes and mechanisms, and biomarkers that account for control of responses to viral pathogens and vaccine efficacy in targeted pig populations. Moreover, transgenic and cloning technologies are likely to enable researchers to produce pigs expressing novel genotypes that will help to affirm gene functions that alter swine viral resistance (Prather, 2007; Schook *et al*., 2008; Matsunari and Nagashima, 2009).

Research using improved swine genome sequence and updated genomic and proteomic tools will reveal novel pathways that regulate antiviral responses and thus open options for novel therapeutics and control alternatives. One factor to consider when selecting for disease resistance is the possibility that the pressure put on pathogens by the presence of genetic resistance in pigs will mean that pathogens will evolve to overcome resistance. The extent of pressure on microbes imposed by pig genetic resistance, and the potential effects of that pressure, are not known today. Will this be different from the pressures due to vaccination and antibiotic treatments?

Throughout this chapter and much of this book, genetic improvement normally implies genetic selection within commercial stocks. For enhanced genetic resistance to disease using marker-assisted selection (MAS) or GWS, this assumes that (i) alleles that confer resistance are present; (ii) these alleles can be identified (or markers linked to them can be identified); and (iii) their allelic frequency can be increased. Genetic selection for disease resistance must focus on improving the ability of the pig to produce an effective response to disease challenge and on simultaneously maintaining production performance. However, if a disease resistance trait has a negative effect on other important traits, then compromises will have to be struck. After genetic markers for resistance are identified, it is important to evaluate them under commercial situations, to determine whether there are any deleterious effects and to analyse whether there is a negative impact on production parameters. Fortunately, the standard selection-index approach incorporating DNA markers has the potential to strike the right compromise.

Because most nucleus swine-breeding populations are maintained with very high health status, selection for resistance to many pathogens that seriously affect commercial herds using quantitative methods may not be practical. Selection using genetic markers or traits that can be measured in uninfected pigs will be preferred. Because of the progress in genomics, genetic prediction can now be based on allele sharing rather than traditional pedigree relationships. This change has opened opportunities to expand genetic selection to a larger number of traits, simultaneously monitoring numerous phenotypes, including health information with growth traits.

The stage is now set for deeper probing of the role of alleles and haplotypes involved in controlling specific antiviral responses, for determining specific genes and their SNPs that are associated with antiviral immune and vaccine responses, and for stimulating critical immune cell subsets and cellular interactions for effective antiviral immune responses. Studies in the next decade will verify whether MAS/GWS for improved viral disease resistance will be effective in commercial settings.

Abbreviations

ASFV, African swine fever virus; BLN, bronchial lymph nodes; CoV, coronaviruse; CSF, classical swine fever; DIVA, differentiation of infected from vaccinated animals; dpi, days post-infection; FACS, fluorescence-activated cell sorting; FMD, foot-and-mouth disease; GWAS, genome-wide association studies; GWS, genome-wide selection; HD, Hampshire Duroc; hpi, hours post-infection; IFN, interferon; IL, interleukin; MAS, marker-assisted selection; OIE, World Organisation for Animal Health; PAM, pulmonary alveolar macrophages; PCV, porcine circovirus; PERV, porcine endogenous retrovirus; PHFD, porcine high fever disease; PHGC, PRRS Host Genetics Consortium; PMWS, post-weaning multi-systemic wasting syndrome; PRCV, porcine respiratory coronavirus; PRRSV, porcine reproductive and respiratory syndrome virus; PRV, pseudorabies virus; QTL, quantitative trait loci; SARS, severe acute respiratory syndrome; SIV, swine influenza virus; SLA, swine leukocyte antigen; SNP, single nucleotide polymorphisms; SVD, swine vesicular disease; TGEV, transmissible gastroenteritis virus; VS, vesicular stomatitis.

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8 Breeding for Resistance to Viral Diseases in Salmonids

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Summary

The salmonids, and in particular the Atlantic salmon (*Salmo salar*) and rainbow trout (*Onchorynchus mykiss*), are 'pioneer species' in modern aquaculture and aquaculture breeding. Viral diseases currently constitute one of the largest challenges to the salmonid farming industry, and resistance to such diseases has become an important part of the goal of many breeding companies. Genetic variation in resistance has been demonstrated for a number of viral diseases both in Atlantic salmon and rainbow trout, with heritability estimates typically ranging from 0.2 to 0.4. The results that are available indicate that genetic correlations between diseases are generally low. For both species and several diseases, response to selection has been experimentally demonstrated. Resistance towards one or more viral diseases is part of the breeding goal of most or all ongoing breeding programmes. Selection is usually based on controlled challenge tests, and since challenge-tested animals cannot be used as brood stock, the within-family component of genetic variation is thus not utilized. Quantitative trait loci (QTL) for resistance towards specific diseases have been searched for, motivated by the need for tools for within-family selection. In Atlantic salmon, resistance towards the viral disease infectious pancreatic necrosis (IPN) was found by two groups to be controlled largely by one QTL. This QTL has been implemented in marker-assisted selection (MAS) by two breeding companies.

Biology and Production Patterns in Aquaculture

The Atlantic salmon (*Salmo salar*) and rainbow trout (*Onchorynchus mykiss*) are two members of the Salmonidae family of fin fishes. They are ecologically important species, prized species among anglers and attractive species for consumption. Due to their easily controllable life cycle, they are also 'pioneer species' within aquaculture. In nature, they are carnivorous species that spend their first years in fresh water, and then migrate to the

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sea following the adaptation process known as smoltification. After a year or more at sea, the fish find their way back to their home river to spawn. This life cycle is mimicked in aquaculture, where the fish are reared in fresh water for the first 6 to 12 months, and then transferred to sea cages for 'growout'. (This production cycle applies to Atlantic salmon and large sea-running rainbow trout; portion-sized rainbow trout are reared in fresh water throughout their life cycle.)

Other salmonid species exist that share the above-mentioned characteristics. However, Atlantic salmon and rainbow trout are by far the most common species in aquaculture and, hence, the ones that are targeted by genetic selection programmes. This chapter will focus primarily on these two species (general aspects of breeding for increased disease resistance in fish have previously been reviewed by Chevassus and Dorson (1990), Fjalestad *et al*. (1993) and others).

From a breeder's perspective, relevant selection traits in salmonids are high growth rate, delaying the age of sexual maturation past the harvest size, filet colour and other quality traits, and resistance to diseases. During the past 10–15 years, resistance to diseases has come to be an increasingly important part of the breeding goal of many breeding programmes. In particular, viral diseases have been given increased attention.

Viral Diseases in Salmonid Species

Salmonids in culture are under attack from a range of pathogens, including bacteria, viruses and parasites. The high densities of fish found in aquaculture promote the outbreak of virulent pathogens, and the aquatic environment itself facilitates the spread of these pathogens from site to site. Today, most bacterial diseases are held at bay by effective vaccines. Viral diseases, on the other hand, continue to cause large losses, both financially and in terms of animal lives. Here, we will consider those viral diseases that are being targeted by artificial selection in breeding programmes, i.e. those viral diseases that are considered most problematic by the industry.

Viral haemorrhagic septicaemia (VHS)

VHS affects many species of fish, and has caused problems for rainbow trout aquaculture for several decades. The disease is caused by generalized infection with VHS virus (synonym: Egtved virus), a virus belonging to the genus *Novirhabdovirus* within the family *Rhabdoviridae*. Clinical signs of acute infection are oedema, haemorrhagic anaemia, exophthalmia and osmotic imbalance. VHS can also occur in a nervous form, manifested by severe abnormal swimming behaviour. The virus is frequent in freshwater rainbow trout culture in continental Europe, and has also occasionally hit sea-water farms in France and Denmark. The disease is absent from salmonid culture outside this region.

Infectious haematopoietic necrosis (IHN)

IHN is caused by another rhabdovirus, and affects *Onchorhyncus* and *Salmo* species during the early life stages. The disease is endemic among salmon and trout (farmed and wild) on the west coast of Canada and the USA. During recent decades, the disease has also spread to continental Europe. Infection is often lethal due to the impairment of osmotic balance, and clinical signs include darkening of the skin, anaemia, oedema and haemorrhages.

Infectious pancreatic necrosis (IPN)

The IPN virus, a double-stranded RNA virus, was the first virus affecting fish to be identified. To date, it remains the best-characterized fish virus. The virus affects Atlantic salmon, rainbow trout and many other species. In an aquaculture context, the disease affects Atlantic salmon at the fry stage (i.e. shortly after first feeding) and at the post-smolt stage (i.e. shortly after transfer to sea water), and rainbow trout at the fry stage. Clinical signs include darkening pigmentation, distended abdomen and abnormal swimming behaviour. The IPN virus is probably present on most Atlantic salmon farms in Europe, mortality rates ranging from zero to 90%. Due to its ubiquitousness, the virus is one the most important loss factors in European farming of Atlantic salmon and rainbow trout.

Infectious salmon anaemia (ISA)

ISA was first reported from Norway in the mid-1980s and has since emerged in most salmon-producing countries. Atlantic salmon is the only susceptible fish species known to develop clinical disease, but the ISA virus may survive and replicate in other species. The ISA virus belongs to the *Orthomyxoviridae* family of single-stranded RNA viruses, and is related to the human influenza viruses. The clinical manifestations of ISA comprise circulatory damage with haemorrhages and haemolysis. The disease course is prolonged with low daily mortality, but cumulative mortality may become very high. The disease had a particularly dramatic effect on the Atlantic salmon industry in Chile, where production decreased from 370,000 t in 2007 to 190,000 t in 2009, largely due to ISA and other disease problems.

Pancreas disease (PD)

Pancreas disease affects Atlantic salmon during the sea-water phase, and is present in Scotland, Ireland, Norway, North America, France and Spain. The disease can also affect rainbow trout. The disease is caused by the salmonid alphavirus (SAV), and several subtypes exist. Affected fish are anorexic and emaciated. Internal gross lesions include haemorrhages in the pancreas and in pancreatic fat between the pyloric caeca.

Genetic Variation in Resistance to Viral Disease in Salmonids

Several studies have investigated genetic variation in susceptibility to viral diseases in salmonids, and produced estimates of heritability. Most studies have used controlled challenge tests performed in the laboratory, a few having been done on the basis of field tests. In controlled challenge tests, the test animals are infected through injections of pathogen, by either adding pathogen to the tank (bath challenge) or adding pre-infected animals to the tank (cohabitant challenge). Mortalities are usually recorded on a daily basis, and pathogen loads are often titred in order to obtain mortality close to 50% overall. In field tests, the animals are put in locations where the specific pathogen is prevalent.

Frequently, the dependent variable used is the 'dead/alive' status of individual fish at the end of the test period. Such data have been analysed using linear models, treating observations as normally distributed (although they are binary). In other studies, threshold models have been used in order to account for the binary nature of the data. In a few studies, survival models have been employed in order to take the precise time of death into account (see Ødegård *et al*., 2006, 2007b, for discussions on different statistical models used for analysis of challenge test data).

VHS

Dorson *et al*. (1995) fertilized identical pools of rainbow trout eggs (from 6 females) with milt from 14 different males, and challenge-tested the resulting half-sib groups. During the following 2 years the procedure was repeated, reusing those males that were still alive at that time. A heritability estimate of 0.63 was obtained. Henryon *et al*. (2002) formed 60 full-sib groups from 30 males and 30 females and challenge-tested 50 of these families to obtain a heritability estimate of 0.13. A similar experiment was conducted by the same authors at a later stage, obtaining a heritability estimate of 0.11.

IHN virus (IHNV)

McIntyre and Amend (1978) estimated heritabilities for resistance to IHNV in sockeye salmon (*Onchorynchus nerka*), by performing three separate challenge tests on the same set of 45 full-sib families. Heritabilities estimated from the three tests, in which challenge was applied 30, 60 and 90 days post-hatching, respectively, were 0.31, 0.27 and 0.38.

ISA

Gjøen *et al*. (1997) challenge-tested 171 full-sib groups within 96 paternal half-sib groups, and obtained a heritability estimate of 0.19. The same families were included when Ødegård *et al*. (2007a) analysed a data set derived from ISA

challenge tests performed on 2960 full-sib families belonging to 11-year classes from a Norwegian breeding programme; the heritability estimate was 0.32.

IPN

Guy *et al*. (2006) used data from field challenges, encompassing 197 full-sib families from a Scottish breeding company tested at each of three sites. The heritability estimates ranged from 0.24 and 0.81 across sites, with the pooled estimate being 0.43. This study was later expanded to encompass 1198 additional full-sib groups and four more sites, with a resulting heritability estimate of 0.38 (Guy *et al*., 2009). Wetten *et al*. (2007) analysed data from fry-stage challenge tests performed on eight different year classes from a Norwegian breeding programme, to obtain heritability estimates with a range of 0.17–0.40 and a mean of 0.31. This study also encompassed data from two field outbreaks, one in fresh water and one in salt water. In both field outbreaks, the genetic correlation to the respective laboratory trial was high: 0.83 and 0.78. Differences in resistance to IPN have also been demonstrated between populations, both in rainbow trout (Okamoto *et al*., 1987a,b) and in brook trout (Silim *et al*., 1982).

PD

Norris *et al*. (2008) exposed 150 full-sib groups, offspring of 60 males and 150 females, to a natural PD outbreak in sea water. PD mortalities were collected over a period of 18 months. The heritability was estimated to be 0.21. The study also produced genetic correlations between PD resistance and production traits, the only significant correlations being those between PD and smolt weight (positive correlation) and filet colour (negative correlation), respectively.

Selection experiments and breeding programmes

Several studies have described improvement in resistance towards specific viruses following selection of the most resistant broodstock. In France, one particularly VHS-resistant rainbow trout male was singled out in a challenge experiment. Offspring from this male were used as broodstock, whereupon increased resistance was demonstrated through increased VHS resistance in the resulting 'grandchildren' (Dorson *et al*., 1995).

In Denmark, relatively VHS-resistant broodstock were selected in a challenge test, and used to produce first- and second-generation gynogenetic offspring. (Gynogenesis is the process of producing offspring that have inherited a double complement of one of the mother's gametes, and no DNA from the father; first-generation gynogens are completely homozygous and non-identical, second-generation gynogens are completely homozygous and identical.) The gynogenetic offspring were found to be more IPN resistant than the source population. The gynogenetic families were later used to study the effect of

complement component C3 genotypes (Slierendrecht *et al*., 1996), and MHC class II genotypes (Slierendrecht *et al*., 2001), on VHS resistance.

In Japan, IPN broke out in a rainbow trout hatchery in 1965. Breeding among survivors across several generations resulted in the development of a highly resistant strain (Okamoto *et al*., 1993). This strain was contrasted with an IPN-sensitive strain (Okamoto *et al*., 1987a,b) in 20 independent challenge trials, yielding average mortalities of 4.3% and 96.1% for the high- and low-resistant strains. These two strains were later crosses in order to produce a resource population for QTL mapping (see below).

Several of the ongoing salmonid commercial breeding programmes incorporate resistance to viral diseases in the breeding goal. At least three breeding programmes breed for increased resistance to ISA. At least four breeding programmes select for increased resistance to IPN. With two exceptions, selection is carried out on the basis of data from laboratory challenge tests, the exception being selection based on field trials for IPN and PD resistance.

From these breeding programmes, only one instance of documented genetic progress has been published. Storset *et al*. (2007) compared the performance of two groups in an IPN challenge test, the high-resistance (HR) group descending from 28 families with average mortality of 1.9% in an earlier challenge test, and the low-resistance (LR) group descending from 9 families with average mortality of 85% in the same test. Mortalities were found to be consistently lower in the HR group than in the LR group, even when different strains of the virus and different challenge methods (bath/cohabitant) were used (Storset *et al*., 2007) (Fig. 8.1). Interestingly, Ramstad and Midtlyng (2008) found that vaccination of the LR group resulted in significantly improved survival, whereas for the HR strain, the effect of vaccination was variable and in part non-significant.

Another, more indirect, indication of the efficacy of selecting for increased IPN resistance has also been found: genotyping of a breeding population with markers for a major gene determining resistance to IPN revealed a notable change in allele frequency towards the favourable allele between two consecutive generations (Moen, 2010).

Correlations between diseases

In aquaculture breeding, resistance against individual disease agents is generally regarded as separate traits. Results have indicated that the genetic correlations between resistance to different pathogens are, generally, low. Gjøen *et al*. (1997) reported weekly negative correlations between resistance to ISA and resistance to the bacteria *Aeromonas salmonicida*, *Vibro salmonicida* and *Vibrio anguillarum* (–0.24 to –0.05), but this finding was not supported by Ødegård *et al*. (2007a), who found small, but significant, positive genetic correlations between resistance to ISA and resistance to *A. salmonicida* based on a larger, but overlapping, data set. Kjøglum *et al*. (2008) found genetic correlation close to zero between ISA, IPN and *A. salmonicida* infections, while Henryon *et al*. (2005) found the correlation between resistance to VHS and resistance to the bacteria *Yersinia ruckeri* and *Flavobacterium psychrophilum* to be non-significant.

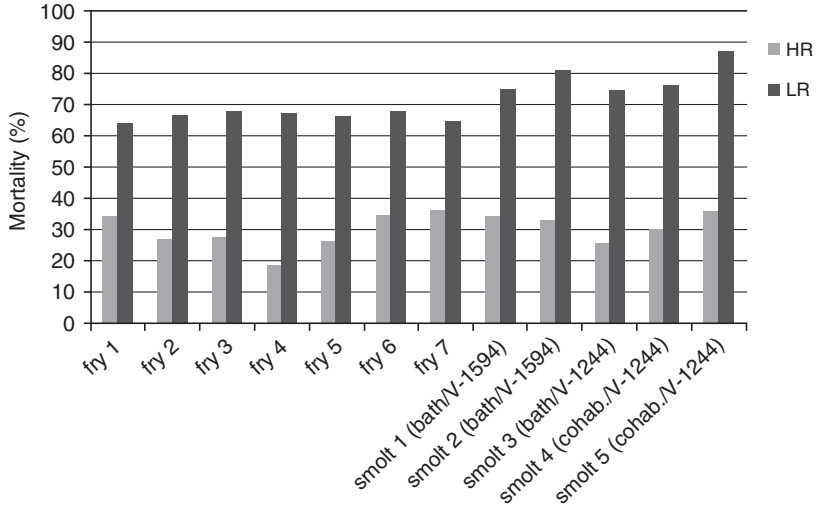

Fig. 8.1. Evidence of genetic progress in resistance to IPN in Atlantic salmon. A high-resistance (HR) group was compared with a low-resistance (LR) group in challenge tests performed at the fry and post-smolt stages, using different challenge models (bath challenge, cohabitant challenge), and different strains of the virus (V-1594, V-1244). The HR group consisted of 28 families derived from parents with high breeding values for IPN resistance; the LR group consisted of 9 families derived from parents with low breeding values for IPN resistance (20).

Individual pathogens may also appear in different serotypes, and isolates within serotypes, that have different characteristics (e.g. Santi *et al*., 2004). Immunity against one isolate does not necessarily guarantee immunity towards another. Indeed, it is often assumed that selection for increased resistance against a strain of a pathogen will lead the pathogen to mutate into a new form against which the host has no defence: an 'arms race' between host and pathogen. However, the (few) results that are available do not indicate any interaction between resistance and viral strain (Storset *et al*., 2007), and there are no published or anecdotal examples of pathogens having mutated into novel strains due to selection on the host.

QTL Mapping and Marker-assisted Selection (MAS) in Salmonids

As is apparent from the above, breeding for increased resistance to viruses in salmonids is largely based on challenge tests in which the survival/no survival status of individual animals is recorded. Due to bio security reasons, it is not considered good practice to breed from animals that have been through challenge tests. Instead, the breeding candidates are given a breeding value that is calculated on the basis of their siblings' performance. In this way, however, no use is made of the within-family component of genetic variation.

Marker-assisted selection (MAS) has been proposed as a means to select directly on breeding candidates. In MAS, animals are selected wholly or partly on the basis of genotypes at DNA markers linked to genes influencing the trait in question (DNA marker genotypes are used as a substitute for genotypes at the trait-influencing genes themselves, since the identity of such genes is generally not known). MAS requires that DNA markers genetically linked to trait-influencing genes are known. These markers can be identified through so-called QTL mapping. In a QTL experiment, sibling groups recorded for the trait in question are genotyped for a number of DNA markers scattered across the genome. Then, the inheritance of marker alleles from parents to offspring is correlated with the offsprings' phenotypes. A QTL mapping experiment identifies a genome region, usually quite large, containing one or more genes influencing the trait in question. This region can be narrowed down by increasing the marker density in the QTL region ('fine-mapping').

QTL mapping for resistance to IHN

A number of studies have been undertaken searching for IHN-resistance QTL in rainbow trout. All of these studies have used crosses between species that differ in their susceptibility to the disease, or crosses between strains of the same species that differ likewise. In such crosses, the proportion of animals being heterozygous at genes influencing the trait is likely to be high, meaning that a large fraction of mapping parents are QTL-informative. Palti *et al*. (1999) identified several QTL in crosses between rainbow trout (*Oncorhynchus mykiss*) and cutthroat trout (*Oncorhynchus clarki*). Later, Barroso *et al*. (2008) found three QTL in another cross between the same species. Khoo *et al*. (2004) identified one suggestive QTL for IHN resistance in an intra-specific cross between a high- and a low-resistance strain of rainbow trout (Khoo *et al*., 2004). Rodriguez *et al*. (2005) found QTL in several linkage groups using another intra-specific cross between high- and low-resistance rainbow trout strains, three of them being found in 2 or more of 20 different mapping parents. None of the QTL identified in these studies were found in more than one study, possibly due to some QTL being false positives or to differences between mapping parents in terms of QTL and marker informativeness. Also, results from inter-species crosses and intra-species crosses are not necessarily comparable since the former detect variation between species and the latter detect variation within species. In addition to the above-mentioned QTL for IHN resistance, significant associations between IHN resistance and the major histocompatibility complex (MHC) class II beta-chain gene (Palti *et al*., 2001) (or loci linked to this gene) have been found.

QTL mapping for resistance to ISA

Moen *et al*. (2004) performed a genome scan on two full-sib families from a Norwegian breeding programme, identifying one significant QTL. This QTL

was later confirmed in a data set consisting of 41 mapping parents and their challenge-tested offspring, with 5 of the mapping parents found to be QTL-heterozygous (Moen *et al*., 2007). In this study, in contrast to the aforementioned IHN studies, 'outbred' families from a breeding programme were used rather than crosses between divergent populations/populations. The benefit of such a strategy is that any QTL identified are relevant for, and hence directly applicable in, the population where they were identified. The downside is that the power to detect QTL is diminished due to a large fraction of mapping parents not being QTL-informative. Significant associations have also been detected between ISA resistance and genes at the MHC class I and class II loci (Grimholt *et al*., 2003; Kjøglum *et al*., 2006).

QTL mapping for resistance to IPN

The strains of rainbow trout displaying high and low resistance to IPN, described by Ozaki *et al*. (see below), were crossed in order to create an F1 generation with high heterozygosity at trait-influencing genes, as well as backcross generations in which these genes would be segregating. Three QTL, located on three different linkage groups, were identified (Ozaki *et al*., 2001, 2007).The case of IPN resistance in Atlantic salmon has provided the most reproducible QTL results in aquaculture so far, leading to a breakthrough with respect to the implementation of QTL in salmon breeding. Houston *et al*. (2008) reported the identification of a QTL responsible for 25% of the phenotypic variation in IPN resistance, based on a genome scan on 10 full-sib families from a Scottish breeding programme, challenge-tested at the post-smolt stage. In an independent genome scan performed at about the same time, encompassing 10 full-sib families of post-smolts from a Norwegian breeding company, Moen *et al*. (2009) identified the same QTL, reporting that it explained 29% of the phenotypic variation in that data set. Moen *et al*. (2009) also tested the QTL in 206 full-sib groups tested at the fry stage, finding that it segregated in 133 out of 300 mapping parents (Moen *et al*., 2009; Moen *et al*., unpublished data). Houston *et al*. (2010) confirmed the effect of the QTL at the fry stage, and reported that the QTL explained almost all the genetic variation at this stage. Taken together, these studies present overwhelming evidence that this QTL, located on chromosome 26 (equivalent to linkage group 21), explains the bulk of genetic variation in IPN resistance at both the fry and the post-smolt stages. Furthermore, they indicate that results from laboratory challenge tests and field trials are comparable, since one study (Houston *et al*., 2008) utilized material from a field trial whereas the other (Moen *et al*., 2009) used material from a laboratory test, and that the QTL must be segregating in quite diverse populations, having been detected in salmon of both Scottish and Norwegian origin. Chromosome 26 is most likely a homologue to one of the rainbow trout chromosomes harbouring QTL for IPN resistance in that species (see above), indicating that the same gene affects IPN resistance in both species (Ozaki *et al*., 2001, 2007).

The large effect and large heterozygosity of this QTL have permitted more in-depth studies. By investigating genotypes in the 133 QTL heterozygous

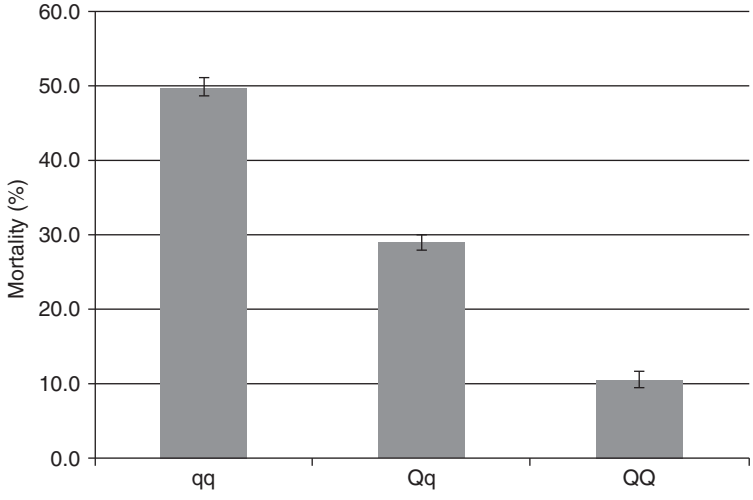

Fig. 8.2. Average survival rate (± standard error) of half-sib groups in a challenge test for IPN resistance (fry stage), categorized according to the genotype of the common parent at a major QTL for IPN resistance (37).

mapping parents, Moen *et al*. could identify haplotypes (combination of alleles at linked markers) associated with the favourable (Q) or the non-favourable (q) allele. This tool permitted the estimation of QTL allele frequencies at the population level, confirming the importance of QTL as a determinant of IPN resistance (Fig. 8.2). The allele frequency of Q was found to have increased from 0.27 to 0.44 between two consecutive generations, a very rare documentation of a large change in allele frequency due to one round of selection (based on phenotype) (Moen *et al*., unpublished data). The tool also permitted the selection of broodstock on the level of the entire breeding population, rather than within a limited set of families with known marker–gene linkage phases (Moen *et al*., 2009).

MAS in salmonids

The QTL for resistance to IPN is the only QTL so far to be used in MAS in a salmonid breeding programme. That QTL, however, is currently being implemented by two breeding programmes, one based in Scotland and the other based in Norway. Both companies reported the use of the QTL broodstock selection for the first time in 2007, although selection was done within a limited number of families in both cases. In 2009, both companies reported that they were testing most or all of their broodstock used for egg production (Newsletter, 2009a,b).

The QTL for IPN resistance is probably not a representative case, since single QTL are rarely found to explain such a large fraction of genetic variance. Also, the lifetime of the QTL as a marker in MAS is likely to be short, since the favourable allele will move rapidly towards fixation even if only conventional selection is applied. This being said, the findings raise the interesting question of how an allele with such an adverse effect on a trait can persist in populations.

It is possible that the low-resistance allele could have beneficial effects on other traits in nature, although the genetic correlations between IPN resistance and other traits recorded by the breeding companies are largely non-significant or positive. However, it is also possible that the QTL is more or less neutral in nature, manifesting itself only in aquaculture, where exposures to virulent forms of IPN are likely to be very different from those in the natural habitats. It is worth noting that, in any case, the availability of tools for determining QTL genotypes of individuals will enable the breeding companies to retain the unfavourable allele in their populations, should they wish to do so in order to retain genetic diversity at the locus.

Conclusion and Future Aspects

This chapter has described five major viral diseases affecting salmonids, diseases that have been targeted by selection experiments and selection programmes. In addition, 'new' diseases are emerging, and could be targeted in the future. For example, heart and skeletal muscle inflammation (HSMI) and cardiomyopathy syndrome (CMS) are emerging diseases believed to have viral aetiologies, currently causing large mortalities in Atlantic salmon aquaculture. Presumably, the not-so-distant future will provide more insight into the genetics underlying resistance to viruses in salmonids (and in general). For example, ongoing and future QTL projects, focusing on different diseases, could reveal whether there are QTL that contribute to resistance against several diseases, as opposed to QTL affecting only resistance to individual diseases. The whole-genome sequence of the Atlantic salmon, a draft of which is due at the end of 2010, will probably boost the search for genes involved in resistance to viral diseases in all salmonids, thus contributing to a more complete understanding of resistance.

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III Bacteria

9 Genetics of Mastitis in Dairy Ruminants

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Summary

Mastitis is a multifactorial infectious disease, mainly caused by bacteria that infect the mammary gland. It is one of the major diseases in dairy ruminants, with respect to industry and public concern, economic impact, zoonotic potential and animal welfare. Genetic control of mastitis has been widely demonstrated. Accordingly, most countries have developed breeding programmes to improve udder health. Most evidence for host genetic variability, and its application for genetic improvement, is principally based on a few basic phenotypic traits related to healthy versus diseased status such as milk somatic cell counts (SCC) and clinical mastitis (CM) occurrence. Additionally, considerable progress has been made in the last decade to scrutinize immune mechanisms and genes that play key roles in the mammary gland defences. Accumulating QTL detection studies have allowed localization of regions of the genome that explain a large part of genetic variability for either SCS or CM traits on almost all autosomal chromosomes. However, resistance to mastitis is a highly complex trait, the genetically determined biological basis of which is mostly unknown. There is still an open field for development of additional relevant phenotypes that can be routinely collected. Further, additional studies are necessary to better understand the genetic basis of mastitis resistance, to predict long-term responses to selection and to develop new tools and strategies for genetic improvement of udder health.

Introduction

Mastitis is an inflammation of the udder caused by a pathogen, which enters the gland through the teat canal and invades the gland cistern. Mastitis is a multifactorial infectious disease; its severity and outcome are highly variable and depend on the host, the environment and the pathogen. Causative micro-organisms are mostly bacteria such as *Staphylococcus* spp., *Streptococcus* spp. or coliforms such as *Escherichia coli*. Clinical mastitis (CM) is especially common in dairy cattle, with frequency estimates ranging from 20 to 40% of lactations affected per year, and rather exceptional in dairy sheep and goat,

 Breeding for Disease Resistance in Farm Animals, 3rd Edition (eds S.C. Bishop *et al.*)

e.g. less than 5%. Subclinical intra-mammary infections (IMIs), however, are widespread throughout dairy cattle sheep and goat herds. In dairy small ruminants, mastitis is the first sanitary cause of involuntary culling, and prevalence of subclinical mastitis is 20–30% per lactation, ranging from 7 to 55% of affected ewes (Bergonier *et al*., 2003a). Classical prophylactic measures, however, including accurate machine milking procedures and hygiene, have not been sufficient to substantially decrease mastitis incidence and avoid dramatic herd outbreaks in recent decades.

Because of its high frequency and biological consequences, mastitis causes major economic losses to the dairy industry. Mastitis is associated with decreased milk production, as reviewed by Hortet and Seegers in dairy cattle (Hortet and Seegers, 1998). Production losses, however, are only a minor impact of mastitis on sustainability; additional costs relate to increased culling rate, penalties on the price of milk, treatment and waste of milk unfit for human consumption in CM cases and prevention. In the previous edition of this book, the authors reported economical evaluations of the disease in the UK to be in the range from £106 to £373 million per annum, including milk losses, treatment and prevention (1996 figures). The average cumulated impact of mastitis (CM and elevated cell counts) in a French study involving 197 herds (1995–1997 figures) was €78 per cow-year or €11 per 1000 l of milk (Seegers *et al*., 2003). By extrapolation, the total annual costs for the French dairy cattle industry would have been about €250 million in 2005. Similarly, from data of five farms (2004–2006) in the USA (Bar *et al*., 2008), the average cost of a CM case was US$71 per cow-year, increasing to up to US$179 at the whole-herd level when full impact on milk losses (64%), treatment costs (28%) and mortality (8%) were accounted for. Regarding dairy sheep and goats, the total EU milk production levels are 2.8 and 2.0 million t, respectively. Making conservative assumptions of a 10% incidence of mastitis in EU dairy sheep and goat flocks as a whole, giving a mean reduction in milk yield in affected animals of 20%, and a wholesale milk price of €650 (sheep) and €580 (goat) per t, the total annual milk production losses to mastitis in small dairy ruminants can be estimated to be in the region of €60 million per annum.

Mastitis also has important negative consequences on dairy ruminant health and welfare, causing udder pain and swelling, discomfort, lethargy, anorexia and even death. The implication of mastitis on food safety relies on the risk of transmission to humans of antibiotic residues (Ruegg and Madison, 2005) and transmission of some mastitis-causing pathogens such as *Staphylococcus aureus*, *Salmonella* spp and *E. coli* O157:H7, especially when milk is unpasteurized (LeJeune and Rajalaâ-Schultz, 2009). In the study of Davies *et al*. (2009), mastitis was one of the major infectious diseases in cattle and sheep with respect to industry and public concern, economic impact, zoonotic potential and animal welfare. Integrating extensive evidence on host genetic variation, authors have placed mastitis as the first-ranked disease for its amenability for genetic studies, with strongly applied perspectives, within and across species in both cattle and sheep.

Indeed, the genetic basis of mastitis resistance was first established in the 1980s in dairy cattle, and became increasingly well documented in the next

decade (Mrode and Swanson, 1996; Heringstad *et al*., 2000; Detilleux, 2002; Rupp and Boichard, 2003). Up-to-date evidence was essentially based on genetic parameter estimation for disease status-related phenotypes such as milk somatic cell count (SCC) and CM occurrence. The earliest genetic studies also provided evidence that the highly successful selection for milk production had probably led to a deterioration in mastitis resistance (Emanuelson *et al*., 1988; Heringstad *et al*., 2007). Accordingly, many countries took advantage of the availability of large-scale routine recording systems and databases for SCC, and for CM in Scandinavian countries (Heringstad *et al*., 2000; Mark and Sullivan, 2006), in the period from 1985 to 2000, to update their breeding objective and incorporate mastitis resistance (Heringstad *et al*., 2000; Miglior *et al*., 2005).

This chapter reviews the state of the art of knowledge on the genetic basis of mastitis resistance in dairy ruminants, with special attention to the most recent research results. It additionally reports on how the information is currently used in breeding programmes for dairy ruminants and discusses the prospects for using genomic selection with both high-throughput single nucleotide polymorphism (SNP) genotyping and targeted quantitative trait loci (QTL) and genes.

Mastitis in Dairy Ruminants

Mastitis is an inflammation of the mammary gland which is characterized by pain, redness, fever and the presence of clots or abnormal appearance of the milk. It is caused in most cases by an IMI. Chronic forms of mastitis are clinically milder and more difficult to detect, although much more frequent.

Aetiology and epidemiology

Basically, IMI is caused by a single bacterial species, although a wide range of bacteria may be isolated from infected mammary glands. Viruses and fungi may colonize mammary glands and produce local signs, but occurrence is far less frequent. Commonly causative microbes are divided into major (such as *Streptococci* and *S. aureus*) and minor pathogens (such as coagulase-negative *Staphylococci* (CNS)) according to the severity of the clinical signs. Mammary bacteria can further be subdivided into: (i) those causing contagious mastitis, which spread from one infected animal to another during milking; (ii) those that are inhabitants of the teat skin and cause opportunistic mastitis; and (iii) those causing environmental mastitis, which are present in the environment and infect the mammary gland from that source.

Numerous reports have made an inventory of the microbes causing acute or chronic mastitis in all three species of domestic ruminant. Among those microorganisms virulence and production of toxins is highly variable, and this modulates the intensity of the clinical signs. The capacity to colonize the teat canal, to adhere to mammary epithelia and to initiate an IMI depends on the

pathogen. The degree of inflammation produced after an IMI is variable, leading to a large range of outcomes from a subclinical to a gangrenous mastitis, depending on the response of the host. However, animal-related factors such as morphological, physiological and immunological factors also determine the severity and the type of an IMI.

In cattle, clinical cases are principally associated with the isolation of one of the following bacteria: *S. aureus*, *E. coli*, *Streptococcus dysgalactiae*, *Streptococcus uberis*, CNS, *Arcanobacterium pyogenes* or *Klebsiella*. Samples with no bacterial growth or with a contamination at sampling generally do not represent more than 10% of the diagnoses. For subclinical mastitis, the rate of positive samples is lower (around 30–40%), and most studies showed a high prevalence of CNS in cattle with elevated SCC (Schukken *et al*., 2009). In heifers there is no relation between the bacterial status before parturition, which is highly variable, and the later development of an IMI, although the presence of an IMI at parturition greatly increases the subsequent risk for the lactating cow (Aarestrup and Jensen, 1997). In dairy sheep and goats, clinical cases are mainly due to *S. aureus*, and occasionally to CNS, *Streptococci* and *Corynebacteria*. In chronic cases, CNS are the most prevalent pathogens as they represent 30–90% of isolates (Poutrel *et al*., 1997; Gonzalo *et al*., 2002). In summary, the aetiology of subclinical mastitis in dairy cows and ewes is closely related, and Gram-positive organisms thus represent 70–90% of isolates in both host species (Contreras *et al*., 1995; Bergonier *et al*., 2003; Bradley *et al*., 2007; Piepers *et al*., 2007). Moreover, the most frequent subclinical mastitis-causing organisms in primiparous females, for both cows and ewes, in the peri-partum and early lactation are CNS. Since the same bacteria are generally not re-isolated later on, they may be considered either as brief mammary infections, teat colonization without fully associated inflammatory reaction, or simple duct contaminations (Daniel *et al*., 1986; Bergonier and Berthelot, 2003; Parker *et al*., 2007).

Recent studies have not revealed any significant shift in the types of bacteria that infect the mammary glands of ruminants during the last decade (Ericsson Unnerstad *et al*., 2008; Sampimon *et al*., 2009), although the frequency of some major pathogens such as *S. aureus* tends, globally, to decrease. In reports from several regions of the world, prevalence of mastitis is close to 50% of milking cattle, and up to 10–25% of quarters (Bradley *et al*., 2007; Olde Riekerink *et al*., 2008). In heifers, prevalence varies widely from 30% to 75% prepartum and from 15% to 45% postpartum (Fox, 2009). The average annual incidence, calculated as the number of clinical cases per 100 cows at risk per year, ranges from 10% to 20% in most herds. In sheep and goats, the frequency of CM is much lower, and does not generally exceed 5% (Bergonier *et al*., 2003). In contrast, chronic mastitis is highly prevalent in these species, leading to large increases in bulk milk SCC (Bergonier *et al*., 2003).

Development of an IMI results from a combination of pathogen- or animal-related risk factors and those linked to environment or management practices, as previously reviewed (Burvenich *et al*., 2003; Moroni *et al*., 2005). In brief, the animal's age, parity, stage of lactation, milk production level, nutritional and metabolic status, and morphological and physiological features of the udder

determine the rate of mastitis occurrence. Breed variability has been described despite numerous confounding factors. When susceptibility to *E. coli* intramammary inoculation was assessed comparing Holstein and Jersey breeds, no difference was observed (Bannerman *et al*., 2008). In sheep, differences in the susceptibility to experimental *Mannheimia* IMI between two Greek breeds of dairy sheep were reported (Fragkou *et al*., 2007). No data are available for breed variability in goats. Factors such as climate, housing system and type of breeding influence the incidence of mastitis in all dairy species. In cattle, design and management of housing system have an impact on the cleanliness of the udder and exposure to environmental pathogens, such as coliforms. Good milking practices and hygiene of the milking parlour are crucial for the control of contagious mastitis.

Host response to IMI

Host resistance to mastitis is a complex trait that involves different components: avoiding entry of the pathogen into the mammary gland, mounting an immune response capable of limiting its development in the udder and clearing the infection, as well as controlling the pathogenic effects of the infection, such as tissue damage. Traditionally, immune responses against IMI are subdivided into innate and acquired immunity, although the two components act in concert and the boundary between them is less clear-cut than often alleged.

Cells associated with innate immunity are essential to the destruction of pathogens. Efficient phagocytes such as neutrophils and macrophages are of key importance in the containment and elimination of the infection. Phagocytic cells originate from the blood and are recruited to the site of infection to provide a first line of defence. The role of neutrophils has been extensively reviewed elsewhere (Paape *et al*., 2003). An early influx of neutrophils, which are able to phagocytose and kill invading bacteria, is considered a determinant for the elimination of coliform mastitis. Neutrophil recruitment is controlled by chemokines such as CXCL8 (IL-8) produced from other sources. However, although their activity can be measured by *in vitro* assays, during infection it is under the control of a series of factors from other cells, such as cytokines. Other soluble factors, such as antibodies produced by plasma B cells and complement, can improve phagocytosis upon opsonization of the bacteria. On the other hand, antibodies may prevent attachment of the bacteria to mammary epithelial cells and block further spreading. Proteins (lactoferrin, lysozyme) and antibacterial peptides help to kill the bacteria that have not been engulfed. Their actions may vary depending on the stage of lactation.

For adaptive immunity, the role of T cells remains poorly understood for mastitis control, and mobilization of a T cell response through vaccination still remains in its infancy for protection against IMI. The major histocompatibility complex (MHC) plays an essential role in the induction and regulation of an acquired immune response (Rothschild *et al*., 2000). Class I MHC molecules are expressed at the surface of all nucleated cells and interact with cytotoxic T lymphocytes (CD8+). In contrast, class II MHC molecules, which are expressed

more by antigen-presenting cells, are involved in antigen presentation to helper T lymphocyte cells (CD4+) and are essential for the development and differentiation of T cells (Rothschild *et al*., 2000).

Recent advances in microarray technology allow exploration of the expression of up to thousands of genes in the context of complex biological functions such as mammary infection. Several recently published applications of this technology to ruminant udder health have mainly addressed the question of differential gene expression in mammary epithelial cells and mammary gland tissue (Schwerin *et al*., 2003; Pareek *et al*., 2005; Strandberg *et al*., 2005; Zheng *et al*., 2006; Jaffrezic *et al*., 2007; Sorensen *et al*., 2007; Lutzow *et al*., 2008; Swanson *et al*., 2009) in milk cells and blood mononuclear cells (Tao and Mallard, 2007), in the context of infection (*in vitro* or *in vivo* models).

Gene expression profiles in bovine epithelial cells showed that CXCL5 genes were significantly over-expressed after stimulation with *E. coli* lipopolysaccharide (LPS) (Pareek *et al*., 2005). Zheng *et al*. (2006) found that after intra-mammary challenge with LPS in mice, most over-expressed genes in mammary tissue and epithelial cells were associated with the innate immune response, including chemokines (CXCL1, CXCL2), acute phase protein SAA3, which may play a role in leukocyte attraction, and LPS-binding protein CD14.

A recent study made by Swanson and colleagues compared the gene expression profile of epithelial cells in culture upon challenge with those of mammary tissue upon infection with *S. uberis* (Swanson *et al*., 2009). The representation of immune-related genes is very different in both situations, indicating the complexity of the mammary response to an infecting pathogen and the coordinated response between resident, recruited and inducible immune factors. Comparative gene expression after intra-mammary experimental challenges of cows with two pathogens (*E. coli* and *S. aureus*) has been implemented and analysed in the context of the EU network European Animal Disease Genomics Network of Excellence (EADGENE) (Jaffrezic *et al*., 2007; Sorensen *et al*., 2007). Results indicated that the pattern of gene expression was significantly different in mammary glands infected by *E. coli* or *S. aureus*, and that *E. coli* modified gene expression more strongly than *S. aureus*, at least at early time points. The most highly upregulated genes following *S. aureus* infection were lactotransferrin and antimicrobial protein secreted in milk, whereas many of the most upregulated genes following *E. coli* infection were associated with influx of neutrophils into the tissues. Furthermore, Lutzow *et al*. (2008) found S100a12, an S100 calcium-binding protein, and Pentraxin-3 (Ptx3) to be upregulated after *S. aureus* infection. The presence of gene products in milk may increase antibacterial properties, thereby helping to resolve the tissue infection. From a case-control study of healthy cows versus cows naturally infected with S. *aureus*, Tao and Mallard (2007) studied expression profiles for a set of 162 immune endocrine genes and reported, among others, upregulation of IL-8 (blood) or IL-17 (milk) gene expression.

Collectively, those studies support the important role of a rapid influx of neutrophils into the mammary gland and the effective and early elimination of pathogens (Paape *et al*., 2003), and strongly support the role of epithelial cells in initiating the inflammatory process (Rainard and Riollet, 2006). In summary,

functional genomics is a promising tool that assists in identifying molecules and pathways that are crucial in the host control of udder health.

Mastitis diagnosis and prediction

Relevant measures of the presence of IMI, also described as diagnostic measures, include bacteriological examination of milk and observation of clinical cases of mastitis. The bacteriological analysis of milk provides precise information on infected quarters and pathogen involved, but it is expensive and time-consuming. Moreover, bacterial shedding is variable and levels may be lower than can be detected by conventional techniques. Indeed, polymerase chain reaction (PCR) tests on bacterial DNA from milk samples could be an attractive diagnostic alternative in the near future. Conversely, cases of clinical mastitis (CM) are much easier to collect on a large scale, but case definition is highly variable from herd to herd and CM refers only partially to udder health problems, especially in small ruminants where frequency of CM is generally lower than 5%. An alternative approach in small ruminants is direct examination of the mammary gland to record mammary abscesses and lesions, which are mainly due to chronic IMI.

The milk somatic cell count (SCC) is an indirect measure, or predictor, of the presence of an IMI. Milk SCC mainly reflects the number of neutrophils that migrate from blood to the mammary gland in response to infection. Increase in milk SCC is therefore desirable to combat invading microorganisms. It gives information about the health status of the udder, and also about the magnitude of the host's inflammatory response. However, numerous factors influence the SCC of infected and non-infected animals, such as the physiological status of the host, infection stage and pathogen. It is therefore difficult to interpret single measures and to define fixed thresholds because distributions of the SCC of infected or non-infected animals overlaps considerably. Some authors suggest that corresponding SCCs relate to two different traits, and have developed mixture models to account for heterogeneity of distribution and to estimate variance components for the SCC in healthy or diseased animals (Detilleux and Leroy, 2000; Ødegård *et al.*, 2003, 2005). Repeated SCC measurements are generally preferred for interpreting the disease status of animals over a given time period. Measured on a monthly basis, the SCC can be interpreted as indicative of the infection's consequences, and a repeatedly elevated SCC can be associated with the presence of chronic mastitis. For statistical analyses, log transformation of the SCC for the purpose of data normalization, such as the transformation resulting in somatic cell scores (SCS) as defined by Ali and Shook (1980), is usually preferred.

Alternative indirect criteria related to inflammatory parameters have been studied, such as lactose content, lactate dehydrogenase and NAGase (Holdaway *et al.*, 1996). Additionally, electrical conductivity of milk relates to the modification of ionic composition (mainly sodium and chloride) during inflammation. It is a promising tool, provided that prediction of an infection is based on within-individual comparison across separate milkings or across quarters

(Hamann and Zecconi, 1998). Specific milking devices might enable routine on-farm recording, with results directly available to the farmer.

Genetic Parameters of Mastitis Resistance and Correlations with Other Traits

Genetic parameters of mastitis resistance

Since the early 1990s, published literature on genetic parameters for mastitis resistance or SCC in dairy cattle has been accumulating, as reported in earlier reviews (Mrode and Swanson, 1996; Detilleux, 2002; Rupp and Boichard, 2003) or in more recent studies in Swedish Holsteins (Carlen *et al*., 2004), Finnish Ayrshire (Koivula *et al*., 2005), Danish Holstein (Madsen *et al*., 2008), Dutch mixed breeds cattle (De Haas *et al*., 2008; Bloemhof *et al*., 2009) and Spanish Holstein (Perez-Cabal *et al*., 2009). Cattle results consistently indicate heritability estimates around 0.15 for lactation average SCS and from 0.05 to 0.14 for monthly test-day SCS. Low heritabilities for CM values, i.e. from 0.02 to 0.04, obtained on the observed binary scale generally increase to around 0.07, with values up to 0.10, when threshold models are applied.

Similar and consistent heritabilities for lactation average SCS have also been reported in dairy sheep. Estimates have ranged from 0.11 to 0.14 in first lactation for various sheep breeds including Spanish Churra (El-Saied *et al*., 1999; Othmane *et al*., 2002), Manchega (Serrano *et al*., 2003), Latxa (Legarra and Ugarte, 2005), French Lacaune (Barillet *et al*., 2001; Rupp *et al*., 2003a), Manech Red Faced (Barillet *et al*., 2008) and Italian Valle del Bellice (Riggio *et al*., 2007). In dairy goats, genetic parameters for lactation average SCS were recently estimated using data from *c*.100,000 French dairy goat lactations (Clément *et al*., 2008). Estimates were 0.20 and 0.24 in the Alpine and Saanen breeds, respectively. In small ruminants there are no data available on genetic parameters for CM.

Most estimates of genetic correlation between SCS and CM in cattle range from 0.30 to 0.80, with an average of 0.70 (review by Mrode and Swanson, 1996, and Rupp and Boichard, 2003; Carlen *et al*., 2004; Koivula *et al*., 2005; Bloemhof *et al*., 2009) or 0.60 from a Scandinavian field data review (Heringstad *et al*., 2000). These reasonably high values suggest a commonality of resistance mechanisms that lead to better resistance to either persistent IMI (continuously high SCC) or acute clinical episodes, despite the fact that those forms of mastitis might be associated with different environmental conditions, different pathogens and a different physiological status of the host.

Genetic parameters for traits other than SCC or CM are scarce. In particular, there are very few data on IMIs assessed by bacteriological testing, which would refer more directly to udder health status. Heritabilities for IMI varied from 0.02 to 0.04 in a study by Weller *et al*. (1992) based on *c*.9800 cows, and were somewhat higher (0.10 to 0.20) for Detilleux *et al*. (1994) and Wanner *et al*. (1998), based on *c*.1200 and 750 cows, respectively. Interestingly, the genetic correlation between SCC and bacterial infection was estimated to be near unity (Weller *et al*., 1992), indicating that SCC and

subclinical infections are essentially the same trait. Recently Sorensen *et al*. (2009) published parameters for pathogen specific mastitis traits, using field data on *c*.200,000 cows in 1747 herds with active disease recording including bacteriological testing for CM. They reported similar, although weakly reliable, correlations of lactation-average SCS with unspecific mastitis (0.69–0.71) or specific mastitis (0.44–0.69) caused by *S. dysgalactiae*, *S. uberis*, *E. coli*, CNS and *S. aureus*.

In addition to mastitis or SCC data, several authors have focused on traits related to the host's defence mechanisms. In particular, genetic aspects of functionality of neutrophils, for which recruitment and activity are essential in the innate defence against udder infection (Sordillo *et al*., 1997; Rainard and Riollet, 2006), have been investigated. A sire effect on *in vitro* phagocytosis of blood neutrophils has been demonstrated (LostrieTrussard *et al*., 1984; Kerhli *et al*., 1991; McDonald *et al*., 1994). Moreover, moderate heritabilities for migration (0.2–0.5) and phagocytosis (0.3–0.7) of neutrophils and for serum complement activity (0.4–0.5), using *in vitro* assays for 137 cows sampled three times around calving, have been reported (Detilleux *et al*., 1994). Some evidence of association with better udder health was given by the finding that animals with low SCC, CM frequency and IMIs tended to exhibit better functionality of neutrophils (Kelm *et al*., 1997). Additionally, antibody-mediated immune response (AMIR), known to be essential in controlling extracellular pathogens such as bacteria responsible for mastitis, has also shown moderate to high heritabilities in dairy ruminants (Wagter *et al*., 2000). Heritability of serum antibody, measured several weeks after vaccination, ranged from 0.32 to 0.64 for ovalbumin and between 0.13 and 0.88 for *E. coli* antigens in peri-parturient cows (Wagter *et al*., 2000).

Genetic relationships of mastitis resistance with other traits

Relationships with milk production traits

The genetic antagonism between mastitis resistance (SCC and CM) and production traits is well documented in dairy cattle, indicating that udder health has been deteriorating as a consequence of selection for production traits. The average genetic correlation between SCC and milk yield is about 0.14, with most values between 0.10 and 0.20 for cattle (reviews by Mrode and Swanson, 1996, and Rupp and Boichard, 2003; Carlen *et al*., 2004; Koivula *et al*., 2005). Genetic correlation estimates with milk yield, however, are quite inconsistent across dairy sheep studies, ranging from antagonistic (Rupp *et al*., 2003a; Riggio *et al*., 2007) to favourable (El-Saied *et al*., 1999; Othmane *et al*., 2002; Serrano *et al*., 2003; Legarra and Ugarte, 2005). In goats, Clément *et al*. (2008) indicated that correlations between SCC and production traits were generally low (from −0.13 to 0.12), with a slightly negative association between SCC and fat yield, e.g. −0.20 and −0.18, respectively. These results suggest that a reduction in SCC can be achieved by selection while still improving milk production in dairy ruminants.

The genetic antagonism between milk yield and CM is noticeably higher (review by Heringstad *et al*., 2000, and Rupp and Boichard, 2003; Carlen *et al*., 2004; Koivula *et al*., 2005) with an average of about 0.35, and most values ranging from 0.20 to 0.55. Possible explanations of this antagonism between udder health and some production components may include an indirect relationship with udder type traits (see next section), selection sweeps for nearby genes that do not necessarily have a related function (Nilsen *et al*., 2009) and they may also involve biological competition between functions for energy and nutrients.

Relationships with udder-type traits and milking ease

Mastitis resistance (SCC and CM) is correlated with several anatomical characteristics of the udder in cattle (reviews by Mrode and Swanson, 1996, and Rupp and Boichard, 2003), sheep (Legarra and Ugarte, 2005) and goat (Clément *et al*., 2008). Udder depth and udder attachment generally show consistent results, indicating that higher and more tightly attached udders are associated with lower SCC. Associations with udder balance, teat length and form have also been reported, but are less consistent across populations, breeds and studies. Such udder-type traits can therefore be considered as easy-to-collect predictors of udder health.

As mentioned in the review by Rupp and Boichard (2003), results on the relationship between udder health and milking ease (or milking speed) are still conflicting. Although a genetic antagonism between milking ease and SCC is consistently and well documented across numerous studies (genetic correlation about 0.40), literature data essentially indicate favourable or almost null estimates of the genetic correlation with CM. As yet, the biological basis of the contrasting relationships between characteristics of milk emission and different udder health-related traits is not well understood.

Relationship of udder health with resistance to other diseases

Inference on relationships between main health disorders in dairy cattle is given by Heringstad *et al*. (2005) from a large Norwegian veterinary field treatment database. These authors estimated favourable genetic correlations of CM occurrence with frequency of milk fever, ketosis and retained placenta, with estimated values ranging from 0.11 to 0.26 in the first three lactations. In agreement with this study, Zwald *et al*. (2004) reported positive genetic associations of mastitis with ketosis (0.17), displaced abomasum (0.08), lameness (0.20) and cystic ovaries (0.11), but no correlation with metritis (−0.01). Positive correlations suggest some commonalty in the genetic basis of various health problems, but may also reflect indirect associations due to the unfavourable genetic correlation with milk production traits.

Resistance to mastitis mainly refers to control of IMIs with extracellular bacteria, with a crucial role for the immune response. However, the immune response is known to mobilize different effectors and pathways according to species and pathogen types. For instance, while type-1 responses predominantly control intracellular pathogens (bacteria and viruses), type-2 responses predominantly control extracellular pathogens such as parasites. From several divergent selection experiments in mice (Mouton *et al*., 1984), poultry

(Pinard-van der Laan, 2002) and pigs (Crawley *et al*., 2005), there is accumulating evidence that different components of the immune response, including type-1 and type-2 responses and resistance to various diseases, are at least partially under independent genetic regulation. Therefore, the question is raised as to whether resistance to mastitis is independent from resistance to other bacterial diseases such as footrot (caused by *Dichelobacter nodosus*, often causing lameness) or paratuberculosis (caused by *Mycobacterium* bacteria), to parasitic diseases (caused by internal nematodes or *Toxoplasma gondii*) or to viral diseases (e.g. Maedi-Visna, the caprine arthritis-encephalitis virus) relevant to ruminant species (Davies *et al*., 2009). These questions have yet to be rigorously addressed.

Molecular Basis for Mastitis Resistance

QTL detection studies

Accumulating QTL detection studies have allowed localization of regions of the genome that explains a large part of variability for udder health traits, as reviewed earlier (Rupp and Boichard, 2003). Most recent studies in cattle, based on genome scans using microsatellite markers, are summarized in Table 9.1. The table includes reference to meta-analyses conducted in the Holstein breed comparing studies in France and Germany (Bennewitz *et al*., 2003) and in the USA (Ashwell *et al*., 2004), as well as in Scandinavian breeds (Lund *et al*., 2007). Based on these studies, a large number of QTL have been detected above the chromosome-wide significance level of 5% (n = 80), for either SCS or CM traits. Udder health QTL were localized on almost all autosomal chromosomes (with the exception of BTA12, 17, 28), with a convergence of evidence on BTA5, 6, 7, 9, 11, 14, 15, 18, 23. Existence of QTL for mastitis resistance, e.g. SCS, has also been demonstrated in dairy sheep (Rupp *et al*., 2003b; Gutierrez-Gil *et al*., 2007).

Results summarized in Table 9.2 for dairy sheep are, to some extent, consistent with cattle results. Gutierrez-Gil *et al*. (2007) found a QTL on OAR20, equivalent to the BTA23 region evidenced by Holmberg and Andersson-Eklund (2004), 30 cM distant from the MHC gene cluster. Suggestive common QTL were found in two independent sheep studies on OAR6 and OAR18 (Table 9.2). Regarding OAR6 results, dairy cattle QTL were quite distant from the equivalent chromosomal region on BTA6 (Table 9.1). For OAR18, however, results may indicate commonality with cattle results for SCS on BTA21. Finally, the reported QTL for SCS on OAR14 was located close to the QTL for SCS on BTA18 (Schrooten *et al*., 2000; Lund *et al*., 2007). In contrast to cattle and sheep, no information exists on chromosomal regions controlling mastitis resistance in the goat.

Several chromosomal regions (BTA5, 6, 8, 9, 11, 14, 18) suggested existence of QTL for both SCS and CM traits. The Finnish, Danish and Swedish cattle studies were the only ones to rely on recording systems for both SCS and CM traits. Accordingly, based on a joint analysis of the three Nordic designs,

Table 9.1. Published QTL for mastitis resistance in dairy cattle, tabulated by bovine autosomes (BTA).

BTA	Trait	Position (cM)	Significance	Reference
1	SCS	59	***	Schulman *et al*. (2004)
	SCS	126	*	Rodriguez-Zas *et al*. (2002)
2	SCS	99	***	Bennewitz *et al*. (2003)
3	SCS	109	***	Schulman *et al*. (2004)
	CM	104	*	Kungland *et al*. (2001)
	SCS	171	*	Schrooten *et al*. (2000)
4	SCS	43	***	Zhang *et al*. (1998)
	CM	39	*	Kungland *et al*. (2001)
5	SCS	7	*	Holmberg and Andersson-Eklund (2004)
	SCS	36	**	Rodriguez-Zas *et al*. (2002)
	CM	24; 58	***	Lund *et al*. (2008)
	SCS	53	**	Lund *et al*. (2008)
6	CM	35	***	Kungland *et al*. (2001)
	CM	117	*	Lund *et al*. (2008)
	SCS	126	*	Lund *et al*. (2008)
7	SCS	39	*	Ron *et al*. (2004)
	SCS	60	**	Rodriguez-Zas *et al*. (2002)
	SCS	61	*	Van Tassel *et al*. (2000)
	SCS	67	**	Aswhell *et al*. (2004)
	SCS	107	*	Kuhn *et al*. (2003)
8	SCS	38	*	Lund *et al*. (2008)
	CM	40	*	Kungland *et al*. (2001)
	SCS	54	*	Kungland *et al*. (2001)
9	CM	10	***	Lund *et al*. (2008)
	CM	71	**	Holmberg and Andersson-Eklund (2004)
	CM	76	*	Lund *et al*. (2008)
	SCS	97	**	Holmberg and Andersson-Eklund (2004)
	CM	72	**	Lund *et al*. (2007)
	SCS	104	**	Lund *et al*. (2007)
	SCS	125	*	Boichard *et al*. (2003)
10	SCS	86	**	Boichard *et al*. (2003)
	SCS	49	*	Kühn *et al*. (2003)
11	CM	26	*	Holmberg and Andersson-Eklund (2004)
	SCS	45	***	Holmberg and Andersson-Eklund (2004)
	SCS	62	**	Lund *et al*. (2007)
	SCS	65	***	Schulman *et al*. (2004)
	CM	123	***	Lund *et al*. (2008)
13	SCS	0	**	Rodriguez-Zas *et al*. (2002)
	SCS	58	*	Lund *et al*. (2008)
	SCS	91	*	Zhang *et al*. (1998)
14	SCS	21	*	Zhang *et al*. (1998)
	CM	25	**	Schulman *et al*. (2004)
	SCS	51	**	Lund *et al*. (2007)
	CM	90	**	Kungland *et al*. (2001)
15	CM	14	*	Lund *et al*. (2008)
	SCS	40	***	Boichard *et al*. (2003)

Continued

Table 9.1. Continued.

BTA	Trait	Position (cM)	Significance	Reference
	SCS	42	*	Rodriguez-Zas *et al*. (2002)
16	SCS	51	**	Rodriguez-Zas *et al*. (2002)
18	SCS	56	**	Lund *et al*. (2007)
	SCS	70	*	Schrooten *et al*. (2000)
	SCS	80	*	Rodriguez-Zas *et al*. (2002)
	SCS	111	***	Schulman *et al*. (2004)
	CM	111	***	Schulman *et al*. (2004)
	SCS	117	**	Kuhn *et al*. (2003)
19	SCS	50	***	Bennewitz *et al*. (2003)
20	SCS	0	*	Rodriguez-Zas *et al*. (2002)
21	SCS	33; 84	*	Rodriguez-Zas *et al*. (2002)
	SCS	51	***	Schulman *et al*. (2004)
	SCS	90	*	Boichard *et al*. (2003)
22	SCS	48	***	Lund *et al*. (2008)
	SCS	80	**	Ashwell *et al*. (2004)
23	SCS	19	*	Boichard *et al*. (2003)
	SCS	20	*	Reinsh *et al*. (1998)
	SCS	41	**	Ashwell *et al*. (2004)
	SCS	52	*	Lund *et al*. (2008)
	SCS	67	*	Holmberg and Andersson-Eklund (2004)
24	SCS	28	***	Schulman *et al*. (2004)
	SCS	41	**	Lund *et al*. (2008)
25	CM	(6.5–15.2)	*	Holmberg and Andersson-Eklund (2004)
	SCS	21	*	Lund *et al*. (2008)
26	SCS	0	**	Ashwell *et al*. (2004)
	CM	53	*	Lund *et al*. (2008)
	SCS	72	*	Zhang *et al*. (1998)
27	SCS	1	***	Schulman *et al*. (2004)
	SCS	8	**	Kuhn *et al*. (2003)
	CM	45	*	Kungland *et al*. (2001)
29	SCS	16	***	Schulman *et al*. (2004)

***Genome-wise significance ($p < 5\%$) experiment-suggestive significance. **Chromosome-wise significance ($p < 1\%$). *Chromosome-wise significance ($p < 5\%$).

Zhang *et al*. (1998): Granddaughter design (GDD) of 14 US AI families with 1794 Holstein sons; 206 markers; multi-marker regression (MMR). Van Tassel *et al*. (2000): GDD of 8 US Holstein families with about 800 sons; 105 markers; single-marker regression with family. Schrooten *et al*. (2000): GDD of 20 Dutch Holstein Friesian families involving 853 bulls; 277 markers; MMR. Kungland *et al*. (2001): GDD of 6 Norwegian cattle families with 285 sons; 288 markers; MMR. Schulman *et al*. (2004): GDD of 12 Finnish Ayrshire families with 491 sons; 150 markers; MMR co-factor analysis (for other QTLs). Boichard *et al*. (2003): GDD of 14 French AI families (Holstein, Normande and Montbeliarde breeds) and 1554 bulls; 168 markers; MMR. Rodriguez-Zas *et al*. (2002): GDD of 8 US AI families with 1065 Holstein sons; 174 MS markers; interval mapping (IM). Kuhn *et al*. (2003): GDD of 16 families involving 872 German Holstein bulls; 263 markers; MMR. Bennewitz *et al*. (2003): Meta-analysis of French and German GDD experiments for 5 common AI families. Ashwell *et al*. (2004): Meta-analysis of US GDD experiments for 6 common AI families; Reg. IM (Aswhell, 1997). Holmberg *et al*. (2004): GDD of 10 Swedish AI families (9 Swedish red and white and 1 Swedish Holstein) with 417 sons; 116 MS markers; Reg. IM. Ron *et al*. (2004): Daughter design (DD) of 11 Israeli Holstein families involving 5221 cows; 94 markers. Reg. IM. Lund *et al*. (2007): Meta-analysis of Nordic designs; 19 grandsires, 672 sons. 61 markers on 5 chromosomes. Lund *et al*. (2008): GDD of 34 Danish families involving 2042 sons; 356 markers; Reg. IM. Multitrait SCS-CM.

Table 9.2. Published QTL for mastitis resistance (SCS) in dairy sheep, tabulated by ovine autosomes (OAR).

OAR	Trait	Position (cM)	Corresponding BTA region (cM)	Significance	Reference
6	SCS (L2)	80	BTA6 (65)	*	Rupp *et al.* (2003b)[a]
	SCS	84	BTA6 (70)	*	Rupp (unpublished data)[b]
12	SCS (L3)	6	BTA16 (13)	**	Rupp *et al.* (2003b)[a]
13	SCS (L1)	28	BTA13 (25)	*	Rupp *et al.* (2003b)[a]
14	SCS	67	BTA18 (50)	*	Rupp (unpublished data)[b]
16	SCS	24	BTA20 (20)	*	Rupp (unpublished data)[b]
18	SCS (L1)	110	BTA21 (90)	*	Rupp *et al.* (2003b)[a]
	SCS	72	BTA21 (60)	*	Rupp (unpublished data)[b]
20	SCS	103	BTA23 (70)	**	Gutierrez-Gil *et al.* (2007)[c]

[a]10 back cross Lacaune Sarda families of 975 ewes; 132 markers. [b]GDD of 18 French Lacaune families with 770 genotyped sons; 162 markers. [c]DD of 11 half-sib Spanish Churra families with 1421 ewes; 181 markers.

Lund *et al*. (2007) investigated the possible pleiotropic effects of QTL using multi-trait analyses. Although the results were not straightforward, they indicated that there might be two linked QTL on BTA9, one that primarily affects CM and another that primarily affects SCS, with some pleiotropic effects in a few families. They could not confirm QTL for CM on BTA11, 14 and 18, and these regions only significantly affected SCS. The pleiotropic effects of the QTL on BTA9 were further confirmed by Sahana *et al*. (2008), using a combined linkage disequilibrium and linkage analysis (LDLA) method with a dense map of the chromosome. In addition, Lund *et al*. (2008) reported a pleiotropic QTL affecting both SCS and CM on BTA5 in their Danish data.

Based on a large dataset comprising *c*.190,000 milk bacteriological analyses, Sorensen *et al*. (2008) further investigated pathogen specificity for udder health QTL on BTA5, 9 and 15. They reported two separate pathogen-specific QTL for SCS on BTA5 (*E. coli* and *S. aureus*), and one pathogen-specific QTL for CM on BTA5 (*S. aureus*). The QTL for CM on BTA9 were found to be specific to *E. coli*. The pathogen specificity of udder health QTL partly enlightens the multitude of results found across studies. In addition to species, breeds and trait definition, QTL might be highly dependent on the pathogen population present in the environment (both the type of pathogen and the infection pressure). It gives some interesting insight into the biological basis of QTL and their pleiotropic effects.

Following genome scan results, intensive fine-mapping work has been conducted by several teams to characterize some of these QTL, particularly on bovine chromosomes BTA6 (Nilsen *et al*., 2009), BTA9 (Sahana *et al*., 2008), BTA11 (Schulman *et al*., 2009) and BTA22 (Sugimoto *et al*., 2006). Sugimoto *et al*. (2006) showed that a polymorphism of the bovine forebrain embryonic zinc finger-like gene (FEZL), located in the region of a QTL for SCC on BTA22, was associated with high and low SCC and, with its transcription activity, led to control of cytokine expression. To our knowledge, this is the first characterized QTL for mastitis resistance. Nilsen *et al*. (2009) utilized a combined LDLA

method, using a dense marker map, to fine-map a QTL on BTA6. From these analyses they found evidence for an association between CM and a polymorphism in the *Mucin* 7 gene, encoding an antimicrobial peptide. The immunoglobulin J chain (IGJ) gene, encoding a peptide involved in the primary immune response, which is located close to the *Mucin* 7 gene, was another strong candidate for this QTL. As mentioned by the authors, the fact that the QTL was close to the casein gene cluster may partly explain the unfavourable genetic correlation observed between mastitis resistance and production traits in many studies and, if correct, this link may potentially be disrupted using markers.

Promising results are expected from the use of high-throughput SNP genotyping techniques and use of linkage disequilibrium to achieve very fine-mapping, ultimately leading to the discovery of causal mutations. Genome scans will benefit from newly released commercial chips, including tens or hundreds of thousands of SNP markers; for example, the Infinium BovineSNP50 BeadChip from Illumina (San Diego, California) and an equivalent Ovine SNP50 BeadChip, driven by the collaborative efforts of the International Sheep Genome Consortium (ISGC) (www.sheephapmap.org). Regarding goats, the Institut National de la Recherche Agronomique (INRA), in collaboration with the French goat industry, aims to provide a low-density SNP chip (1536) to allow genome scans and to investigate udder health in this species in the near future. Because of similarity between the bovine, ovine and caprine genome and physiopathology of mastitis, it is likely that results from one species may prove useful in other species.

Major histocompatibility complex genes

Because of the key role of MHC molecules in the immune response, and because genes encoding those molecules are highly polymorphic, they have been extensively studied as candidate genes for disease resistance in recent years (Buitkamp *et al*., 1996; Rothschild *et al*., 2000; Stear *et al*., 2001). MHC genes are located on BTA23 and OAR20 in cattle and sheep, respectively, and provide functional candidates for QTL detected in the corresponding chromosomal regions (see above). Independently from QTL detection studies, several dairy cattle studies have reported a relationship between bovine MHC (bovine lymphocyte antigen (BoLA)) class I molecules and resistance or susceptibility to mastitis (Weigel *et al*., 1990; Mejdell *et al*., 1994; Aarestrup and Jensen, 1997; Mallard *et al*., 1995) or immune response (Mallard *et al*., 1995). Nearly all of the most recent studies, however, have focused on the exon 2 of the Class II DRB3 locus because of its high level of polymorphism and because it encodes the antigen-binding site of MHC molecules (Rothschild *et al*., 2000). Several alleles have been found to be associated with mastitis resistance, as measured by decreased SCC and mastitis frequency (Dietz *et al*., 1997; Kelm *et al*., 1997; Starkenburg *et al*., 1997; Sharif *et al*., 2000; Kulberg *et al*., 2007; Rupp *et al*., 2007) and with increased or decreased antibody or cell-mediated immune response (Rupp *et al*., 2007). A summary of published results is shown in Table 9.3. Interestingly, the associations between BoLA DRB3.2 alleles (DRB3.2 *3,

*24 and *22) and immune responses tended to be in opposite sign for the two antibody versus cell-mediated immune response traits examined (Rupp *et al*., 2007), in agreement with the hypothesis that they represent opposite type 1 and type 2 immune responses. From published literature (several hundreds of animals), however, only alleles DRB3.2 *11 and DRB3.2 *22 were consistently associated with resistance or susceptibility to mastitis, respectively, in at least three independent studies. Unfavourable associations of alleles DRB3.2 *22, *23 and *8 with udder health (Starkenburg *et al*., 1997; Sharif *et al*., 2000; Kulberg *et al*., 2007; Rupp *et al*., 2007) were in agreement with the finding of Sharif *et al*. (2000) that some common amino acid motifs in the antigen-binding groove of corresponding BoLA molecules are involved in susceptibility to mastitis. Most results of associations between given BoLA alleles and phenotypes were conflicting across studies, however, making it difficult to draw conclusions about the direct causal effect of BoLA alleles on udder health. Genetic background (breed), environment effects (nature of pathogens) or trait definition may explain those discrepancies, but selective sweep, small data sizes or spurious results are also likely explanations. As concluded by Kulberg *et al*. (2007), inconsistency of results across studies advocates considering haplotypes rather than alleles at single BoLA genes. Use of MHC alleles for selection purposes is therefore unlikely, particularly as some alleles could be associated with resistance for udder health, but associated with susceptibility to other diseases.

Other candidate genes

Candidate gene approaches have investigated genes other than MHC, such as those encoding CD18, TLR2 and chemokine molecules. The CD18 gene (BTA1) encodes adhesion molecules expressed on the surface of leucocytes. Homozygous cattle for the deleterious allele exhibit leukocyte adhesion deficiency (Blad) (Nagahata, 2004), which leads to impaired diapedesis of leucocytes, extreme sensitivity in any infection and premature death. However, attempts to show association with udder health in heterozygous animals have not been successful. Currently, other candidate genes are under investigation, such as genes coding for Toll-like receptors (TLR, BTA6) (Opsal *et al*., 2006; Sharma *et al*., 2006) or acute phase proteins (haptoglobin and serum amyloid-A) involved in non-specific innate immune response components during the early stages of mastitis (Schwerin *et al*., 2003; Gronlund *et al*., 2005). Recently, a Canadian team investigated the association of SNP polymorphisms within a few candidate genes with SCS estimated breeding values of 338 Holstein bulls (Leyva-Baca *et al*., 2007, 2008; Pant *et al*., 2008). Pant *et al*. (2008) presented some evidence of an association of polymorphisms within the caspase recruitment domain 15 gene (CARD15) with SCS estimated breeding values. CARD15 (BTA18), similarly to TLR2, is an important pattern recognition receptor that plays a role in the initiation of the immune response against bacteria. Leyva-Baca *et al*. (2007) mentioned a possible association with an SNP within the CCR2 (BTA22) gene, encoding a chemokine

Table 9.3. Significant associations between MHC Class II DRB3 alleles and different indicators of mastitis.

Reference	Genotyped animals	Model	Allele	Effect
Dietz *et al*. (1997)	584 cows	Logistic regression (case control)	DRB3.2*16	Higher acutely elevated SCC
Kelm *et al*. (1997)	137 cows	GSM	DRB3.2*3	More IMI with minor pathogens
			DRB3.2*8	More CM
			DRB3.2*16	Higher SCC
			DRB3.2*24	More IMI with major pathogens
			DRB3.2*11	Less CM
			DRB3.2*23	Less CM
Starkenburg *et al*. (1997)	186 cows	GSM	DRB3.2*7	Higher SCC
			DRB3.2*22	Higher SCC
			DRB3.2*24	Higher SCC
			DRB3.2*24	More CM
			DRB3.2*3	Lower SCC
			DRB3.2*8	Less CM
			DRB3.2*16	Lower SCC
Sharif *et al*. (2000)	835 cows (Holstein)	GSM	DRB3.2*23	More severe CM
			DRB3.2*16	Lower SCC
Sharif *et al*. (2000)	835 cows (Holstein)	Fisher test (case control)	DRB3.2*8	More CM with *Staphylococci*
			DRB3.2*22	
			DRB3.2*23	
			DRB3.2*24	
Rupp *et al*. (2007)	328 cows (Holstein)	GSM	DRB3.2*8	More CM
			DRB3.2*22	Higher SCC
			DRB3.2*23	Higher SCC
			DRB3.2*3	Lower SCC
			DRB3.2*3	Less CM
			DRB3.2*11	Lower SCC
Kulberg *et al*. (2007)	452 cows (Norwegian red)	GSM with relationship matrix	DRB3.2*22	More CM
			DRB3.2*26	More CM
			DRB3.2*7	Less CM
			DRB3.2*11	Less CM
			DRB3.2*18	Less CM
			DRB3.2*24	Less CM

GSM, gene substitution effect model; SCC, somatic cell counts; IMI, intra-mammary infection; CM, clinical mastitis.

receptor molecule. Furthermore, authors indicated an association between estimated breeding values for SCS and one SNP in the CXCR1 gene (not localized on the bovine genome) that encodes a receptor expressed on the surface of neutrophils that interacts primarily with CXCL8 (IL-8), the most potent chemoattractant for neutrophils.

Combining mRNA differential display techniques for infected and non-infected bovine udders, and co-localization in QTL regions, Schwerin *et al*. (2003) identified four genes, AHCY (BTA13), PRKDC (BTA14), HNRPU (BTA16-OAR12) and OSTF1 (BTA8), as potential candidates involved in mastitis resistance. These genes were located close to most recent QTL results from cattle and sheep (Tables 9.1 and 9.2). Recently, Bonnefont *et al*. (2009) studied gene expression profiles in neutrophils of sheep divergently selected on SCS. They reported a list of 15 differentially expressed genes between susceptible and resistant sheep after experimental challenge. Among those genes, a few were localized close (<15 cM) to bovine mastitis resistance QTL for SCS and were relevant candidates for further investigation.

Breeding for Improved Mastitis Resistance

The Scandinavian countries were the first to consider udder health in their breeding objectives for dairy cattle, as early as the 1980s (Heringstad *et al*., 2000). More recently, in the last decade, many other countries similarly modified their breeding objectives for dairy cattle (Mark and Sullivan, 2006; Miglior *et al*., 2005) and sheep (Rupp *et al*., 2002; Barillet *et al*., 2007) in response to increasing consumer concern for better animal health and food quality, and also to maximize profitability by reducing production costs.

Selection criteria and udder health index

An accurate selection criterion must be a relevant biological trait that is genetically well correlated with mastitis resistance, exhibits sufficient genetic variability and has operational properties such as being easy and cheap to measure on a large scale. Accordingly, SCC is the most widely used criterion to achieve better udder health. Indeed, repeated SCC data are routinely recorded for individuals as part of milk-recording schemes and are stored in large databases in many countries. Similar large-scale recording has also existed for CM in Scandinavia for more than 20 years (Heringstad *et al*., 2000). Therefore, Sweden, Finland, Norway and Denmark focus on CM in their udder health index and additionally consider SCC. As reviewed by Heringstad *et al*. (2000), single-trait evaluation is carried out in Finland and Sweden, and then weighted by an udder health index. Denmark uses a multi-trait model to increase accuracy of estimated breeding values for mastitis resistance. Norway used solely CM information until recently, but now also includes SCS. In the Scandinavian countries, to obtain a high accuracy of sires' estimated breeding values, the low heritability of CM is counterbalanced by large progeny group sizes. Implementation of similar large-scale recording is under way in several other countries such as France, and genetic evaluation for CM in cattle, in addition to SCC, will probably be generalized worldwide in the future. Accuracy of estimated breeding values for udder health is also improved by including information on early predictors, particularly udder

conformation (udder depth), for example in Denmark, Canada and the Netherlands (Miglior *et al*., 2005).

For small dairy ruminants, classical quantitative selection is more challenging. Only a few dairy populations worldwide, mainly located in the Mediterranean region (such as the French and Spanish sheep and goat populations, and Italian sheep populations) or in North America, have the required organization to allow development of large-scale recording, genetic evaluation and breeding programmes. Among major obstacles are the lack of genetic ties between flocks and limited use of artificial insemination (AI). Additionally, recording costs per animal, relative to potential income, for traits other than production are prohibitive in many situations. The French Lacaune breed is the only small ruminant dairy breed selected for increased udder health (Rupp *et al*., 2002). Genetic evaluations for lactation mean SCS have been run since 2002, based on a simplified recording system for SCC (Rupp *et al*., 2002), and are implemented in the same way as that for fat and protein content. Genetic evaluations for SCC are also under way for the Alpine and Saanen French dairy goat, and these have benefited from long-term recording for SCC based on the dairy cattle system and a strong industry organization.

Inclusion of mastitis resistance in multi-trait selection

Breeding objectives for dairy ruminants aim to improve not only udder health but also production traits (milk fat and protein yield, fat and protein content) and other functional traits such as durability (conformation, longevity) and reproductive success. Miglior *et al*. (2005) reported the relative weight that was given to udder health in the total merit indexes for 12 countries among 15 that had provided data. The average relative weight was 6.3%, and it was especially important in Denmark, France, Israel, the USA (Net Merit index) and the UK (TOP index), i.e. 14%, 12.5%, 11%, 9% and 8%, respectively. In those countries the economic weight for udder health, compared with production in the total merit index, was 1:2.4, 1:2.0, 1:7.0, 1:6.1 and 1:6.5 in Denmark, France, Israel, the USA and the UK, respectively. This review did not include data from Norway, Finland and Sweden, which have considered udder health since the early 1980s (Heringstad *et al*., 2000). In Norway, the economic weight for mastitis compared with milk yield in the total merit index was 1:4.5 in 1980 (Svendsen, 1999). From 1990 it became 1:1.6 and later 1:1.0. In the French Lacaune dairy sheep, the current relative weight for SCC is 25% in the total merit index ISOL, with relative weights of SCS compared with production of 1:2.0. The ISOL also includes udder conformation, with a relative weight of 25% (Barillet *et al*., 2007). At current selection intensities, such a combination is expected to reduce SCS by one genetic standard deviation over 10 years.

Overall, with the main emphasis on production traits in breeding objectives of dairy cattle, the general aim is to stabilize SCS or even decrease it, but a deterioration of CM could be anticipated, especially when CM is not considered in addition to SCS. Response for CM in small ruminants, which have a low frequency, is of less importance.

Practical effects of selection for mastitis resistance

In Norway, CM has been included in the breeding objective for 30 years. Norwegian cattle are therefore a relevant population in which to assess the effectiveness of long-term selection for mastitis resistance. Based on estimated breeding values of Norwegian red sires by birth-year of daughters, Heringstad *et al*. (2000, 2007) reported a flat trend from 1978 to 1990 and a favourable genetic change of −0.27% CM per year afterwards, e.g. from approximately 20.5% in 1990 to 19.0% in 1996. These authors mentioned the increased relative weight placed on CM in the total merit index in recent years as an explanation of the trend change in the 1990s. Similarly, in the Finnish Ayrshire population, where CM and SCC have been included in the total merit index since 1986 and 1993, respectively, Juga *et al*. (1999) reported a favourable genetic decrease of 0.15% CM per year for bulls born from 1983 to 1993. In parallel, Norwegian geneticists (Heringstad *et al*., 2007) evaluated the response of CM-based selection in a long-term experiment using AI bulls, started in 1989. After five generations of single-trait selection, the authors reported a decrease in CM frequency from 15% to less than 5%, again demonstrating that considerable improvement can be achieved in mastitis resistance provided CM data are used and sufficient selection intensity is applied. Interestingly, these authors also reported favourable correlated selection responses for ketosis and retained placenta, two common diseases of dairy cattle.

To evaluate the effect of SCC-based selection, a single-generation divergent selection based on SCS-estimated breeding values of parents was conducted in sheep (Rupp *et al*., 2009). Based on preliminary results on a first cohort of 84 sheep, the authors confirmed a large difference between lines for SCS of about three genetic standard deviations. A significant decrease of CM, mammary abscess and IMIs caused by various pathogens (measured by repeated milk bacteriological tests) was observed in the low-SCS line when compared with the high-SCS line (Rupp *et al*., 2009). Experimental infection with two different *Staphylococci* further indicated reduced milk losses and bacterial counts, after challenge in low-versus high-SCS ewes (Bonnefont *et al*., 2009). In standardized challenge conditions, selection for decreased SCS therefore seemed to be correlated with a better ability to control the development of bacteria and to limit the consequences of infection and inflammation. Overall results of the sheep experiment provided evidence that SCS-based selection may help to improve host resistance to mastitis and decrease the frequency of both clinical and subclinical IMIs.

Including molecular information for mastitis resistance in breeding programmes

As for many functional traits with a low heritability, which are difficult to select with conventional approaches, molecular information within classical breeding programmes through gene- or marker-assisted selection (MAS) is expected to increase the accuracy and therefore the efficiency of the selection process (Schulman and Dentine, 2005; Guillaume *et al*., 2008) In practice, the use of MAS is still limited

worldwide for mastitis resistance, probably because of the large number of QTL regions described, the small proportion of the genetic variance explained by each QTL, their large confidence intervals and poor across-study validation.

A dramatic breakthrough is expected from progress in high-throughput genotyping technologies for thousands of SNP markers throughout the whole genome. Consequently, fine-mapping of QTL will probably be much accelerated in future years. As an example, updating the French QTL design (Boichard *et al*., 2003) with additional sires (n = 2837) and genotyping with the Illumina 'bovine SNP50' chip, Fritz *et al*. (2008) reported a significant increase in the number of QTL detected, e.g. 15–30 QTL for SCS in each of three breeds, namely Holstein, Normande and Montbéliarde. Most of the significant QTL for SCS were located on BTA2, 3, 4, 6, 8, 9, 12, 15, 16 and 29 and were included in the MAS in 2008 (Fritz *et al*., 2008). As an alternative to the use of a number of targeted regions with fine-mapped (or fully characterized) QTL, genomic selection may be based on the prediction of breeding values from whole-genome marker information (Meuwissen *et al*., 2001). It is especially relevant in the mastitis case, where the infinitesimal model, hypothesizing a large number of genes each with a small effect, is likely to be more appropriate than a model with a few major genes. The first application of genomic selection for mastitis resistance in dairy cattle has recently been reported (Raadsma *et al*., 2008), indicating that an animal's genetic merit can be predicted (accuracy of 0.47 in the results presented) by SNP markers genotyped from DNA samples at birth before any phenotypic information is available.

While GS is expected to lead to major genetic improvements in sustainability traits for dairy cattle, there are probably fewer prospects for genomic selection for functional traits in small ruminant species, except for the biggest and most organized populations, such as the French Lacaune breed. As mentioned earlier, the smaller size and disrupted structure of populations and breeds make it difficult to implement the necessary 'training' step for genomic selection, e.g. estimation of SNP effects for mastitis resistance, before the 'predictive' step can be implemented. For across-breed and across-environment evaluations, SNP arrays denser than are currently available are likely to be needed. Costs might also make genomic selection prohibitive in small ruminants, unless they drop dramatically. There is, however, a more promising scenario in the identification of very closely linked genetic markers and causative mutation for use in gene selection or MAS.

Questioning the breeding goals and selection criteria

Currently, selection strategies for improved udder health are based on decreasing milk SCC and, in Scandinavian countries, on the reduction of CM occurrence, as a tool to decrease both subclinical and clinical IMIs caused by various bacterial species. The question has been raised, however, whether SCC can drop too low in the long term. Indeed, it has been stated that decreasing milk SCC to very low levels could impair the healthy cow's capacity to combat IMI, as some of the resident cells in the milk, such as macrophages, are essential in initiating the inflammatory process in response to intra-mammary invading

pathogens. The first evidence of the potential role of mammary epithelial cells in neutrophil recruitment (Rainard and Riollet, 2006) somewhat moderated the latter concern. Additionally, early studies, mainly based on experimental challenges, showed that moderate cell counts in milk play a protective role in the defence of the mammary gland (Schalm *et al*., 1964; Schukken *et al*., 1994). Finally, several herd-level studies showed high CM incidence risk in low-SCC herds (Elbers *et al*., 1998; Waage *et al*., 1998; Beaudeau *et al*., 2002). However, evidence of a favourable association between udder health and low SCC has also been given. First, the genetic relationship between (log-transfomed) SCC and CM is linear and no intermediate optimum has been observed (McDaniel and Adkinson, 1993; Philipsson *et al*., 1995; Cranford and Pearson, 2001). Second, two studies have shown the lowest risk of mastitis for cows with the lowest observed SCC, in contrasting herd epidemiological situations (Rupp and Boichard, 2000; Rupp *et al*., 2000). In addition to the divergent selection experiments in dairy sheep and cattle described above, these results suggest that diminishing SCC to the lowest observed values is a relevant means to decrease the level of infected animals. Furthermore, given the present levels of SCC and CM frequencies, as well as current selection pressure put on mastitis resistance (CM and SCC), milk SCC is not likely to decrease dramatically in the near future.

Considering SCC as a single continuous trait whatever the environment and level may, however, be too simplistic in the long term. There is likely to be much benefit in redefining traits describing mastitis resistance (Bishop, 2008). Accordingly, two alternatives to SCC lactation mean or test-day SCC have recently been developed. De Haas *et al*. (2008) suggested deriving new traits based on patterns of peaks in SCC and proportion of test-day SCC above various thresholds. The objective was better use of SCC information, rather than using a simple mean, in order to capture pathogen-specific mastitis, recovery and (essentially) account for CM when data are not available for genetic evaluation. On the other hand, other authors (Ødegård *et al*., 2003, 2005) suggested defining liability to mastitis based on mixture models for SCC data. Indeed, SCC data at a population level might reflect a mixture of different traits depending on whether animals are infected or not, and depending on the mastitis-causing pathogens. Such an approach provides a means to disentangle genetic relationships between, and the biological significance of, baseline SCC in healthy udders and SCC in infected animals. Madsen *et al*. (2008) implemented a linear mixture model to Danish SCC data in order to derive a breeding value for liability to putative mastitis rather than solely for decreased SCC. Interestingly, they reported a genetic correlation of 0.61 between baseline SCC and SCC in infected animals, indicating that cows having high SCC when healthy are also more likely to exhibit high SCC when infected. However, the difficulty in disentangling the true biological baseline SCC from the response SCC might result in an overestimation of the latter correlation.

Further consideration of pathogen-specific response in SCC can be taken into account in mixture models, provided bacteriological data are available. It might prove useful to address the question of customized bacteria-specific selection, given that current selection strategies are probably relevant in improving

resistance to the most frequent udder pathogens, e.g. *Staphylococci*, in the current epidemiological situation.

Conclusions

Genetic control of udder health in dairy ruminants has been widely demonstrated. Accordingly, most countries have developed breeding programmes to improve udder health. Most evidence for genetic variability and application for genetic improvement, however, are principally based on a few basic phenotypic traits related to the healthy versus diseased status, such as milk SCC and CM occurrence. Therefore, they give only a few clues about components of the genetic control of resistance, the universality of these mechanisms in various epidemiological situations (environment, pathogen) and their validity over time. There is an open field for the development of additional relevant phenotypes that can be routinely collected.

On the other hand, considerable progress has been made in the last decade to scrutinize immune mechanisms and genes that play key roles in mammary gland defences, but their function is highly complex and this is still a large field for investigation. Studies combining different approaches such as genetics and QTL detection, immunology and epidemiology are necessary better to understand the genetic basis of udder health, to predict long-term responses to selection and to develop new tools and strategies for the genetic improvement of udder health.

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10 *Salmonella* in Chickens

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Summary

There are over 2000 *Salmonella* bacterial species, many of which have very wide host–species ranges. Infection with *Salmonella* in chickens generally progresses through three distinct phases: first, invasion via the gastrointestinal tract; second, establishment of a systemic infection in internal organs; and third, one of three distinct outcomes in the final phase. In the final phase, *Salmonella* infections in chickens may be completely resolved by effective immune mechanisms, or may cause acute and severe pathology, even death, or may result in an asymptomatic and chronic carrier state. Carrier birds can shed bacteria into eggs and into the environment, causing vertical and horizontal transfer of bacteria, and may serve as a source of microbial contamination of poultry meat and eggs. Thus, in addition to negatively impacting chicken health, *Salmonella* contamination of poultry products represents a threat to human health. With strong consumer preferences and, in some countries, legislation limiting the use of antimicrobial drugs in poultry production, enhancing genetic resistance to *Salmonella* is an attractive component of a comprehensive programme to control disease in poultry. The chicken's response to *Salmonella* is complex, with many cellular components, including early-responding macrophages and heterophils, and utilizes innate immune mechanisms, Th1 cytokine production and protective cellular and humoral immune responses. There is strong evidence for genetic control of many facets of host response to *Salmonella* infection. As reviewed in this chapter, distinct lines of chickens have been characterized with different responses to *Salmonella*, several specific genes have been demonstrated to have structural or expression variation associated with salmonellosis or *Salmonella* carrier state, quantitative trait loci (QTL) for *Salmonella* response have been identified by genomic scans and transcriptional analyses have elucidated key genes and pathways that are altered in response to infection with *Salmonella*. Therefore, although there is much remaining to be discovered, a foundation of knowledge already exists about the genetic control of resistance to *Salmonella* in chickens that will enable the use of genetic approaches to enhance bird health and reduce microbial contamination of poultry products.

 Breeding for Disease Resistance in Farm Animals, 3rd Edition (eds S.C. Bishop *et al.*)

Introduction

Hazards of *Salmonella* to chickens and humans

Some species of *Salmonella* bacteria are highly pathogenic in chickens, while other species cause little response in the host birds, which can then become asymptomatic carriers. Because the asymptomatic birds are maintained in production flocks, these birds with subclinical salmonellosis can transmit the zoonotic bacteria into the human food chain. Bacteria with asymptomatic presence in poultry may represent food-borne pathogens that cause disease in humans (Doyle and Erickson, 2006). Gast and Holt (1998) showed that chicks infected with *Salmonella* immediately after hatch can be persistently colonized to maturity, when the bacteria are shed vertically to infect table or hatching eggs, or horizontally to infect other hens. This susceptibility to long-term colonization may be due to the relative hyporesponsiveness that young chicks exhibit towards *Salmonella* (Holt *et al*., 1999) and can be induced by exposure to low or high doses of bacteria (van Immerseel *et al*., 2004).

Poultry salmonellosis is an important food safety concern. *Salmonella enterica* Serovar Enteriditis (*Salmonella enteritidis* (SE)) is a major food-borne pathogen, accounting for 82% of food-borne salmonellosis (Kramer *et al*., 1998). Contaminated eggs are a leading cause of *Salmonella* food poisoning, and poultry flocks with high levels of SE pose the largest threat of egg contamination (Kramer *et al*., 1998). In the USA, there are an estimated 1.4 million cases of human infection with *Salmonella* species each year (Mead *et al*., 1999). Recent case-control studies identified chicken consumption as a major risk factor in SE infections (Kimura *et al*., 2004). To achieve reductions in human illnesses, effective preharvest interventions are needed (Singer *et al*., 2007).

Systemic salmonellosis is primarily caused by *Salmonella gallinarum* and *Salmonella pullorum*, which do not have flagella, and thus are not recognized by TLR5 (Chappell *et al*., 2009). This is hypothesized to be one of the reasons why these serovars do not induce a strong inflammatory response in the gastrointestinal track and limit the infection to this site; infections instead often progress to systemic salmonellosis. *Salmonella typhimurium* and SE do induce an inflammatory reaction in the chicken host, which often limits the infection to the gut in a chronic carrier state, thus presenting the hazard of potential contamination of poultry products. A strong T cell response appears to be crucial in resistance to salmonellosis (Beal *et al*., 2005).

The biological impact of bearing a microbial burden also reduces growth and reproductive performance (Klasing and Korver, 1997). This reduced performance can occur even in typical production environments in which *Salmonellae* are present but are not causing clinical disease symptoms. Thus, control of *Salmonella* presents special challenges to the poultry industry. Greater understanding of the genetic mechanisms controlling bacterial and cellular interactions could be applied to decrease bacterial colonization, faecal shedding and manure contamination, which would significantly improve preharvest food safety, as well as animal health and efficiency of production.

The complexity of the host defences against *Salmonella* gives many opportunities for genetic approaches to improve host resistance to salmonellosis or carrier state of *Salmonella*.

Feasibility of host genetic approaches to reduce *Salmonella* pathology and carriage in chickens

To determine the feasibility of using modulation of host genetics to help improve resistance to salmonellosis, it is essential to establish that there is at least partial genetic control of *Salmonella* response traits. Estimated heritability of resistance to caecal carrier state, measured by enrichment culture of *Salmonella* persisting in the caecum after challenge, was 0.20 in laying hens, but only 0.06 when using the frequency of positive caeca (Berthelot *et al*., 1998). Heritabilities of the number of bacteria persisting in internal organs ranged from 0.02 to 0.29 (Girard-Santosuosso *et al*., 2002). Chick mortality after *Salmonella* challenge was estimated to have heritabilities of 0.14 and 0.62, respectively, for sire and dam components; spleen contamination of laying hens at 0.47 and 0.13, and caecal contamination of laying hens at 0.24 and 0.53 (Beaumont *et al*., 1999). Reported heritability estimates for antibody response to *Salmonella* range widely from 0.03 to 0.26 (Kaiser *et al*., 1997; Beaumont *et al*., 1999). Because vaccine antibody level has a negative genetic correlation with caecal colonization, vaccine antibody is a useful biomarker in improving resistance to colonization (Kaiser *et al*., 2002b). Measuring antibody response is less costly with regard to time and materials than measuring bacterial colonization (Kaiser *et al*., 1998). Line differences or heritability estimates of several parameters of *Salmonella* response, therefore, indicate that genetic selection to improve resistance to *Salmonella* carrier status and salmonellosis is feasible.

Genetics of Resistance to *Salmonella* in Chickens

Gene-centric approaches

Biological candidate genes: variation in structure

Many genes are associated with the multifactorial traits of resistance to *Salmonella* species, and most have a relatively small individual effect on the disease phenotypic variation, which is consistent with complex control of the disease by many genes (Hasenstein *et al*., 2008). Factors such as definition of host resistance phenotype (antibody, mortality, systemic or enteric colonization), genetic line, population structure, number of birds and serovar of *Salmonella* studied may result in variation detected in results across studies. However, several genes have shown a robust association with *Salmonella* response across a variety of conditions (Wigley, 2004; Calenge *et al*., 2010).

SLC11A1 (NRAMP1). Comparative genomics strategies have been effectively applied to identify genes controlling salmonellosis resistance in chickens, because bacterial resistance was studied previously in the mouse as a model organism. Chicken homologues of major loci controlling natural resistance of mice to infection with *S. typhimurium* were examined as candidate genes. Variation in natural resistance-associated macrophage protein 1 (NRAMP1), now called SLC11A1 as a member of the solute carrier gene family, and Tenascin C (TNC) were shown to account for 33% of the differential resistance in *Salmonella*-induced mortality in a backcross (BC) population of inbred lines (Hu *et al*., 1997). The TNC locus is closely linked to the gene formerly known as lipopolysaccharide (LPS), now known as TLR4, which binds LPS, a major component of membranes of Gram-negative bacteria such as *Salmonella*. The NRAMP1 association with *Salmonella* resistance has been expanded to many different traits of host response to *Salmonella* in other chicken populations (Beaumont *et al*., 2003; Kramer *et al*., 2003; Liu *et al*., 2003), as has been the TLR4 association (Beaumont *et al*., 2003; Leveque *et al*., 2003; Malek *et al*., 2004). Success in the comparative genomics approach provided a foundation to select additional candidate genes to test. Positional candidates based upon genomic position include CD28 and VIL1 in the NRAMP1 region (Girard-Santosuosso *et al*., 1997). The CD28 gene was found to be associated with *Salmonella* enteric infection (Malek *et al*., 2004), and VIL1 with visceral infection (Girard-Santosuosso *et al*., 2002).

MAJOR HISTOCOMPATIBILITY COMPLEX (MHC). The MHC, because of its crucial role in antigen processing and presentation, has been investigated for its role in response to *Salmonella*. In a series of 12 B-complex congenic lines, line differences occurred in morbidity and mortality after *Salmonella* challenge (Cotter *et al*., 1998). Given the congenic nature of the lines, these differences should be attributable to genes within the MHC region, although the specific gene(s) are not known. Structural variation in the MHC class I gene, tested in experimental crosses, has also been associated with resistance to *Salmonella* colonization in the spleen (Liu *et al*., 2002) and with level of antibody response to a commercial *Salmonella* bacterin vaccine (Zhou and Lamont, 2003).

CYTOKINES. Cytokines are the essential communication molecules secreted by cells of the immune system and other tissues, and are primarily responsible for initiating or modulating pro- and anti-inflammatory responses, or directing the immune response to a predominantly Th1 or Th2 response. Chemokines, in concert with their receptors, regulate immune cell circulation and recruitment to sites of infection. The transforming growth factor β (TGFβ) gene products have cytokine-functional properties. Genetic variants in TRAIL (tumour necrosis factor-related apoptosis-inducing ligand), TGFβ3 and interleukin-10 (IL-10) have been associated with bacterial load in caecal content and/or spleen (Malek and Lamont, 2003; Ghebremichael *et al*., 2008). A study of several commercial and regional Dutch meat-type lines of chickens found associations with single-nucleotide polymorphisms (SNPs) in IL-2, TGFβ2 and TGFβ4 genes with bacterial load in the caeca (Kramer *et al*., 2003).

TOLL-LIKE RECEPTORS (TLR). The TLR are a family of transmembrane proteins that are involved in generic identification of pathogens by sensing pathogen-associated molecular patterns (PAMPs) and inducing innate immune responses. Utilizing a comparative genomics approach, Leveque *et al*. (2003) postulated that TLR4, known to influence *Salmonella* infection in a mouse model, would also have a role in *Salmonella* response in chickens. The TLR4 gene was cloned, and TLR4 polymorphisms were demonstrated to be linked to resistance to infection with *S. typhimurium* (Leveque *et al*., 2003). Because of the specific interaction of TLR5 with SE flagellin, this TLR is also a strong candidate to have polymorphisms associated with *Salmonella* response (Keestra *et al*., 2008). The interactions of TLRs and *Salmonella* are likely to be complex, as combinatorial recognition of bacterial lipoproteins and peptidoglycan by chicken TLRs has been demonstrated, and it is suggested that the chicken TLRs may have evolved to form heteromultimers that function in recognition of combinations of microbial patterns (Higuchi *et al*., 2008).

β-DEFENSINS. The genes encoding the antimicrobial β-defensin peptides of the chicken are tightly clustered on chromosome 3, with two recombination hot spots in the cluster (Hasenstein and Lamont, 2007). These antimicrobial proteins are important factors in the innate immune response against bacteria (van Dijk *et al*., 2008). Gene fragments were sequenced from each of 13 β-defensin genes from three birds of each of two advanced intercross lines (broiler by layer; broiler by Fayoumi) and a mean of 14.8 SNPs per kilobase identified (Hasenstein and Lamont, 2007). Then, one allele-specific SNP per gene was genotyped to test statistical associations with SE colonization after challenge. Among the 13 β-defensin genes evaluated, 4 (AvBD3, -11, -12, and -13) were associated with bacterial load in caecal content and 1 (AvBD5) with *Salmonella* load in spleen tissue (Hasenstein and Lamont, 2007).

APOPTOSIS. Apoptosis, or programmed cell death, is an important functional feature of the immune system. Genes in apoptotic pathways include caspase 1 (CASP1) and inhibitor of apoptosis 1 (IAP1). A CASP1 SNP was associated with *Salmonella* persistence in the spleen and caecum in an experimental cross (Liu and Lamont, 2003) and in the liver and caecum in commercial broilers (Kramer *et al*., 2003). The IAP1 gene SNP variation was associated with bacterial load in spleen (Liu and Lamont, 2003) and caecum (Kramer *et al*., 2003).

IMMUNOGLOBULINS. Antibodies (antigen-specific immunoglobulins) are an important feature of the acquired immune response. SNPs in the polymeric immunoglobulin receptor (PIGR) were shown to be associated with bacterial burden in spleen and caecal content (Ghebremichael *et al*., 2008), and SNPs in the immunoglobulin light chain (IGL) with level of antibody response to *Salmonella* vaccination (Malek *et al*., 2004).

OTHER GENES. Involvement in pathways that are hypothesized to be important in host response to *Salmonella* is a criterion for selection of other candidate genes to study. The myeloid differentiation protein-2 (MD-2) gene product

interacts with the TLR4 receptor on the cell surface and, therefore, based on the reported role of the TLR4 gene in *Salmonella* infection (Leveque *et al*., 2003), the MD2 gene was examined; SNPs in MD2 were associated with persistence of *Salmonella* colonization in the caecum (Malek *et al*., 2004). Genetic variation in candidate genes cluster of differentiation 28 (CD28), map kinase-activated protein kinase 2 (MAPKAPK2;), inducible nitric oxide synthase (iNOS) and prosaposin (PSAP) has also been associated with various traits of response to *Salmonella* (Lamont *et al*., 2002; Kramer *et al*., 2003; Malek and Lamont, 2003; Malek *et al*., 2004; Ghebremicjael *et al*., 2008).

Most candidate-gene experiments, because of linkage disequilibrium in the populations tested, cannot exclude the possibility that the causal gene could be a nearby gene rather than the specific gene studied. Supporting lines of evidence, such as confirmation in independent populations, with QTL scans, by gene expression data or from comparative genomics, adds confidence in the detected gene-resistance associations. Gene expression studies with contrasts of *Salmonella*-challenged versus unchallenged groups, or resistant versus susceptible infected animals, are currently revealing transcriptional differences in genes that may be active in pathways controlling resistance; these are discussed in the following sections.

Biological candidate genes: variation in expression

CYTOKINES. Cytokines are key communication molecules between host cells in defence against pathogens. Infection of chickens with *Salmonella* induces expression of multiple chemokines and cytokines in a variety of tissues. Three distinct chicken breeds (broiler, Fayoumi and Leghorn) were evaluated for messenger ribonucleic acid (mRNA) expression levels at 2 and 18 h post-inoculation of day-old chicks with SE (Cheeseman *et al*., 2007). *Salmonella* inoculation significantly increased splenic IL-18 and interferon-gamma (IFN-g) expression. Breed effect was significant for CXCLi2, IL-10, IL-12a and CCLi2 mRNA expression in the spleen, and for IL-12a, IL-12b, IL-18 and CCLi2 mRNA expression in the caecum. These results support a role for breed genetics influencing cytokine mRNA expression in young chickens and may potentially explain some generalized immune response differences between breeds.

Heterophils are a 'first-responder' cell type in response to *Salmonella* infection and, therefore, the transcriptional changes induced by the bacteria may be of crucial importance in initiating an effective immune response (Swaggerty *et al*., 2005). Swaggerty *et al*. (2004) examined cytokine gene mRNA levels in four lines of broilers, including two parental lines and their F1 crosses. The heterophils from the two phenotypically resistant lines, after isolation and treatment with SE, had higher levels of pro-inflammatory cytokines IL-6, IL-8 and IL-18 and lower levels of the anti-inflammatory TGFβ4 than the two susceptible lines. In a study of diverse chicken lines, heterophils from lines with a history of commercial selection for meat or egg production (broiler and Leghorn birds, respectively) had early heterophil responses to SE that differed from the native Egyptian Fayoumi line (Redmond *et al*., 2009). Stimulation with SE *in vitro* increased the expression of pro- (IL-6 and GM-CSF) and anti-inflammatory (IL-10 and TGF-b4) cytokine mRNA in the Fayoumi line,

whereas the broiler and Leghorn line heterophils had decreased or not changed. The findings illustrate the potential value of native lines to provide biodiversity to enhance innate health in commercially selected poultry. Macrophages are another early-responder cell type in the immune system. Wigley *et al*. (2006) isolated macrophages from blood of resistant and susceptible lines of chickens, and demonstrated that the cells produced cytokines with different profiles of time and level between the two genetic lines. The resistant line produced cytokines more rapidly and at higher levels, including IL-18, which suggests a role for Th1 adaptive immunity in protective responses.

Although there is variation in results among the studies that were conducted with a variety of genetic lines, challenge species of *Salmonella*, timing and cells or tissues assays, a generally consistent picture emerges: IL-1α, TNFα, IFN-γ, IL-12, IL-18 and IL-15 generally appear to be associated with a protective role, and IL-4 and IL-10 with inhibition of host defences against *Salmonella*.

TOLL-LIKE RECEPTORS (TLRS). As microbial pattern recognition receptors (PRRs) that will initiate the immune response after detection of PAMPs, the expression of TLRs has been the object of many studies. Expression level of TLR4, the receptor for bacterial LPS, differed after SE challenge between chicken lines with different levels of resistance to *Salmonella* (Sadeyen *et al*., 2006). In an assessment of the early effects (2 and 18 h after oral inoculation of day-old chicks) of *Salmonella* infection in three distinct genetic lines on splenic mRNA expression, TLR2, TLR4 and TLR5 were all downregulated in Leghorns, TLR2 and TLR5 were downregulated and TLR4 was upregulated in broilers, and only TLR5 was downregulated and both TLR2 and TLR4 were upregulated in Fayoumi birds (Abasht *et al*., 2009). Earlier, Iqbal *et al*. (2005) demonstrated a role of TLR5 in recognizing flagella, thus potentially restricting the spread of those *Salmonella* species bearing flagella. It is therefore interesting to note the downregulation of TLR5 in all three genetic lines assessed in the Abasht *et al*. (2009) study, which might be beneficial to protect host cells from overstimulation by bacterial flagellin (Abasht *et al*., 2008).

TLR1 gene expression was decreased in response to *S. typhimurium* infection in leukocytes isolated from peripheral blood of broilers at 6 h to 2 days after infection (Meade *et al*., 2009). *In vitro* exposure of isolated heterophils to a variety of TLR ligands induced differential expression of several cytokines in lines with differential resistance to *Salmonella* (Kogut *et al*., 2006). Based upon the upregulation of TLR15, an avian-unique TLR, after *S. typhimurium* exposure of chicken fibroblasts, Higgs *et al*. (2006) suggested that this newly identified TLR might play an important role in salmonellosis. Recently, this was verified when TLR15 expression was shown to differ in heterophils isolated from two broiler breeder chicken lines that are relatively resistant or susceptible to *Salmonella* (Nerren *et al*., 2009).

β-DEFENSINS. Antimicrobial peptides are an important component of the innate immune response to pathogens; therefore, the expression of avian β-defensin (AvBD) genes early after infection may be expected to be important

in initiating a strong defence reaction. AvBD gene expression (AvBD3, AvBD10 and AvBD12) was significantly increased in response to *S. typhimurium* infection in leukocytes isolated from peripheral blood of broilers at 6 h to 2 days after infection (Meade *et al*., 2009). Two inbred chicken lines, differing in caecal bacterial carriage, were evaluated for expression of several genes, with the most marked differences in expression between lines being the β-defensins (AvBD1 and AvBD2) (Sadeyen *et al*., 2006). The higher levels (up to tenfold higher) of AvBD gene expression occurred in the line with the lowest bacterial carriage levels. To determine the cellular source of AvBD1 and AvBD2 in intestinal tissues, embryonic intestinal cells were isolated from these two chicken inbred lines that were phenotypically diverse for *Salmonella* carriage and expression level of these two defensin genes. Intestinal cells from the two different lines differentially expressed AvBD1 and AvBD2. Additionally, *Salmonella* interfered with AvBD2 expression in the cells from the susceptible, but not the resistant, line (Derache *et al*., 2009).

Genome-wide approaches

Genome-wide approaches involve identification of quantitative trait loci (QTL), and through fine-mapping moving from genomic region to positional candidate genes. Initially these were achieved using low-density genome scans; however, with the advent of the sequenced genome, high-density genome scans using possible SNP arrays are now possible.

Low-density scans; identification of supernumerary aleurone layers 1 (SAL1)

Use of low-density whole-genome scans using markers such as microsatellites, which were state of the art at the time the studies were conducted, has identified many QTL regions controlling *Salmonella* resistance and other response phenotypes (Abasht *et al*., 2006). In using genome-wide approaches, the study is not constrained by knowledge of specific genes, but rather seeks to identify regions of the genome with an effect on the traits of interest. Low-density scans are generally insufficient to resolve the QTL interval down to the scale of identifying a few underlying candidate genes. However, they provide a foundation for additional study in promising regions of the genome.

A notable success of low-density genome scans is the identification of a QTL named SAL1. Using a BC population of resistant and susceptible parental inbred lines (Lines 6_1 and 15I, respectively), Mariani *et al*. (2001) genotyped birds with extreme high or low bacterial counts in the spleen. They identified significant linkage between spleen colonization and a region defined by genetic markers on chromosome 5. This region was named SAL1. Fine-mapping of the chromosomal location of the QTL found a very strong effect in the region near the creatinine kinase B (CKB) and dynein, cytoplasmic, heavy polypeptide 1 (DNCH1) genes, accounting for 50% of the parental variation difference. The distance of almost 50 cM between salmonellosis resistance locus 1 and the *Salmonella* resistance QTL linked to marker ADL0298 (avian disease lab

#0298 marker) (Kaiser and Lamont, 2002) makes them unlikely to represent the same locus, although both influenced the resistance trait of bacterial colonization in the spleen in other studies. Wigley *et al*. (2002) expanded studies on the SAL1 region to demonstrate that effects were detectable both *in vivo* and *in vitro*. The specific gene in the SAL1 QTL associated with resistance is not yet identified, but recent fine-mapping of the SAL1 region has revealed strong positional and biological candidate genes (Fife *et al*., 2009). A sixth-generation BC of defined resistant and susceptible lines was genotyped with 37 fully informative SNPs to refine the SAL1 locus to a position between 54.0 and 54.8Mb on the long arm of chromosome 5. The mapped interval spans 14 genes, of which 2 are functional candidates for *Salmonella* response: AKT1 (protein kinase B (PKB)) and CD-27-binding protein.

Tilquin *et al*. (2005) conducted low-density genome scans for *Salmonella* resistance QTL using both F2 and BC populations formed from Lines N (a different resistant line) and 6_1, and measured phenotypes of cloacal and caecal bacterial carrier state. They confirmed an association of the SAL1 region with enteric carrier state. New QTL regions were also identified at significant or suggestive levels on chromosomes 1, 2, 5 and 16, including QTL with effects as large as 37.5% of the phenotypic variance. Although the two tested inbred lines are considered to have the same MHC haplotypes, the QTL on chromosome 16 maps to the MHC and may represent variation in a non-classical MHC-region gene. The chromosome 5 QTL maps near TGFβ3, which was demonstrated in candidate-gene studies of independent populations to be associated with spleen bacterial colonization (Kramer *et al*., 2003) and bacterial level in the caecal contents (Malek and Lamont, 2003).

Additional QTL regions for diverse phenotypes related to *Salmonella* response in chickens have been identified in focused studies with small numbers of markers, and confirmation and/or fine-mapping of previously identified QTL regions for *Salmonella* response traits has been done. The following examples are illustrative of principles that may apply over a larger range of investigations. The work of Yunis *et al*. (2002) illustrated the feasibility of identifying QTL for response to both SE and *Escherichia coli*. This supports the concept that some of the identified QTL have broad effects across a range of bacterial pathogens, which will be advantageous in genetic selection to improve resistance to multiple food-safety pathogens. Kaiser *et al*. (2002a) identified QTL for antibody response to a commercial vaccine against *Salmonella* vaccine. This demonstrates the feasibility of studies that record a specific immune-response trait correlated with resistance and one that can be collected on the live bird, not requiring biosecure facilities or sacrifice of the birds to obtain the phenotypic data for QTL identification. Five previously identified QTL (Tilquin *et al*., 2005) were further examined in a more comprehensive population set from the original experimental population and also in populations derived from a commercial line (Calenge *et al*., 2009). Two QTL were verified in each population, although only one QTL in the SLC11A1 region was shared between the populations. This illustrates the utility of experimental

populations to identify QTL regions that are also detectable in commercial populations.

High-density scans for single-nucleotide polymorphisms (SNPs)

The availability of the genomic sequence of the chicken (Hillier *et al*., 2004) and a 2.8 million SNP map (Wong *et al*., 2004) has enabled the production of increasingly higher-density SNP genotyping platforms. Studies have been completed and published on first-generation (~1000–3000 SNP) SNP chips and are now ongoing with SNP panels of up to 60,000 SNPs.

Hasenstein *et al*. (2008) analysed SNPs from the 3072-SNP Illumina panel designed by Hans Cheng (USDA, East Lansing, MI, USA) and colleagues for association with SE burden levels in the spleen and caecum of chickens of two F_8 advanced intercross lines. The advanced intercross lines were developed by crossing broiler males with hens of highly inbred Leghorn or Fayoumi lines, and then intercrossing in subsequent generations. Most of the SNPs in the panel were originally selected to be in gene exons based upon genomic sequence but, after adjustments from the second genome build, SNPs were seen to be located in both exons and introns. Of the approximately 1000 informative SNPs in each line, a total of 21 SNPs were significant for SE bacterial levels. Eight SNPs were associated with spleen bacterial burden and 13 with caecal bacterial burden. The 21 significant SNPs were in or very near 19 genes with diverse reported molecular functions, including apoptosis, cell signalling and DNA repair. AMP-regulated phosphoprotein 21 (ARPP-21) and mutL homologue 1 (MLH1) were the only genes associated with both spleen and caecal bacterial burden. Ten SNPs marked genes in pathways that have been previously associated with immune response to *Salmonella* challenge (TLR signalling, apoptosis and mitogen-activated protein kinase (MAPK) signalling pathways), further supporting the SNPs' role in *Salmonella* response. In addition to identifying 19 new candidate genes for host response to SE in chickens, the significant SNPs may be useful in marker-assisted selection (MAS) programmes for disease resistance.

Fife *et al*. (2010) analysed a BC1 population of Lines 6_1 and N for associations of caecal bacterial levels and 1255 SNPs distributed across the genome. Four QTL were identified: on chromosomes 2, 3, 12 and 25. There is general agreement between multiple, independent genome-wide QTL studies in the location of some of the QTL. The QTL for hardened caseous caecal core identified by Fife *et al*. (2010) at 20.0 Mb on chromosome 2 is somewhat near the several QTL reported by Hasenstein *et al*. (2008) on chromosome 2 between 43.2 and 46.1 Mb for colonization of spleen or caecum, and very near that reported by Tilquin *et al*. (2005) at 22.6 Mb for gut colonization. The QTL on chromosome 3 at 96 Mb in the Fife *et al*. (2010) study is somewhat near the two QTL identified in the Hasenstein *et al*. (2008) study on chromosome 3 at 78.4 and 80.1 Mb for bacterial colonization of caecum and spleen, respectively. The repeated identification of similar genomic regions bearing QTL for related traits in different studies strengthens the case for those regions to be bearing true QTL and warranting detailed fine-mapping to identify the causal genes.

Functional genomics: microarrays

Use of large-scale expression profiling using microarrays is helping to develop a broad picture of the transcriptional differences that occur in response to *Salmonella* infection, some of which are also associated with host response differences between resistant and susceptible birds. From microarray experiments, however, it is generally not possible to separate the expression changes that may be predictive of the outcome of infections from those changes that are simply a result of different treatment effects. None the less, microarrays are a powerful tool to identify genes and biological pathways associated with *Salmonella* infection phenotypes, which can then serve to direct future, targeted studies of gene function and *Salmonella* resistance to identify the causal genes.

IMMUNE-CENTRIC ARRAYS. Immune-centric arrays are ones produced from tissues relevant to the immune response, usually immune tissues or cell lines infected with pathogens, which increases the probability of the array efficiently identifying elements involved in the immune response to infection with *Salmonella*.

Gene expression profiles were compared in chicken intestine of two genetically different chicken lines, harvested 24 h after SE inoculation of day-old chicks (van Hemert *et al*., 2006a), using both a whole-genome oligonucleotide array and a cDNA microarray. The innate immune system and wound-healing genes were upregulated after infection regardless of the line. The lines differed in expression of genes encoding proteins for inflammation, acute phase response, the fibrinogen system and actin polymerization. In a study designed to assess gene expression differences over time after infection of day-old chicks with SE, van Hemert *et al*. (2006b) found differences associated with infection status as well as genetic line. At 1 day post-infection, the slow-growing broiler line had expression differences in genes related to macrophage activation, and the fast-growing line in genes related to T cell activation. Later, at 7–9 days post-infection, the largest number of detectable differences was found in comparing the two lines under control conditions, suggesting innate differences in intestinal function between these lines, which might give rise to differences in resistance to *Salmonella*. Another study measured T cell sub-populations, phagocytic functions of intestinal mononuclear cells and jejunal RNA expression levels after infection of day-old chicks with *Salmonella* (van Hemert *et al*., 2007). Infected and non-infected birds differed in intestinal mononucleocyte phagocytic activity, and the infection-induced changes in T cell numbers showed line-specific responses. More genes were differentially expressed in one line only than were regulated in both lines in response to infection. Thus, genetic line is an important factor in host response to *Salmonella*.

The HD11 macrophage cell line was infected *in vitro* with SE, and transcriptional profiles assayed on a 5K activated macrophage/monocyte array (Lillehoj *et al*., 2007). The chemokine ah294 had the highest expression at all time points (2, 5 and 24 h). Other genes induced by infection included IL-6 and

anti-apoptotic genes. Genes that were downregulated after infection included many that are associated with cell proliferation, adhesion and transcription.

GLOBAL GENE-EXPRESSION ARRAYS. Use of global arrays that broadly represent genes of the host serve as discovery platforms to identify not only the same genes that might emerge as significant on immune-centric arrays, but also the genes and pathways from tissues not well represented on the more limited-scope arrays. Additionally, they can serve as a consistent platform for interrogation of gene expression in experiments directed towards many different physiological systems (growth, development, etc.), thus helping to identify elements of importance across a range of systems in the host.

The chicken 13K cDNA array from the Fred Hutchinson Cancer Research Center (Seattle, Washington) is a global array with transcripts derived from 24 tissue and cell sources (Burnside *et al*., 2005). An assay on this array of spleens harvested at 1 week after oral inoculation of day-old chicks with SE revealed many genes that were significantly differentially expressed between the inoculated and the non-inoculated chicks, as well as between the chicks with high and low bacterial burden (Zhou and Lamont, 2007). Prominent groups of genes that were differentially expressed included cytokines and chemokines, and genes related to apoptosis and T cell function. Using the same 13K microarray, it was found that diverse genetic lines of birds preferentially used different biological systems in their early, innate response to *Salmonella* infection, as defined by transcriptional differences in the spleen at 2 or 18 h after inoculation of day-old chicks. The Leghorn line predominantly uses immune response; the Fayoumi line, apoptosis and non-immune cellular responses; and the broiler line, immune defence mechanisms. This study demonstrates the wide species diversity, and the genetic line specificity, of host transcriptional response to *Salmonella* infection in chickens (S.J. Lamont, Iowa State University, and H. Zhou, Texas A&M University, personal communication, 2010).

The transcriptional response of chicken heterophils from a relatively resistant and a relatively susceptible commercial line, exposed *in vitro* to SE, was studied by Chiang *et al*. (2008). The effect of the genetic line was greater than that of the infection status for a number of differentially expressed genes. However, the susceptible line showed more genes related to immune function to be downregulated than the resistant line. In specific analysis of the immune-related genes, a stronger and upregulated response was seen in heterophils from the resistant line compared with the susceptible line. The upregulated genes included members of the TLR signalling pathway and genes that activate T-helper cells.

Although the various individual studies utilized different global or immune-centric arrays, sources of chicken tissue or cell type assayed, and specific challenge treatment with *Salmonella*, timing, etc., some consistent pictures are emerging for the important pathways associated with response to *Salmonella* (Table 10.1). Prominent candidates for genetic control of *Salmonella* in poultry, as identified through transcriptional profiles, are cytokine, chemokine, TLR and antimicrobial peptide genes, and genes of the apoptotic and T cell functional pathways.

Table 10.1. Gene families and pathways, and representative genes, associated with response and resistance to *Salmonella* in chickens.

Gene family/pathway	Representative genes
Toll-like receptors	MD-2, TLR1, TLR2, TLR4, TLR5
Cytokines	ah294, IL2, IL4, IL6, IL8, IL10, IL18, IFNG, K60, MIP1B, RANTES, SOCS3, TGFB2, TGFB3, TGFB4
Apoptosis	Bcl-x, CASP1, Fas, IAP, TRAIL, TNF-R1
Antimicrobial peptides	AvBD2, AvBD3, AvBD5, AvBD11, AvBD12, AvBD13
Cell-surface antigens	CD3, CD40, MHC2A, MHC2B

Conclusions

Genetic selection for improved immunity and resistance traits to *Salmonella*, which can be accomplished after sufficient genetic markers are defined, has the potential to improve poultry health and production efficiency, as well as reducing food-borne pathogens in the human food chain (Lamont, 1998; Lamont *et al*., 2003). One of the most straightforward approaches would be to conduct MAS with SNPs in genes of large effect on the desired traits. Strong candidate genes include SLC11A1, MHC, several cytokines, beta defensins, TLRs and genes in apoptotic pathways. The fine-mapped region containing SAL1 also provides a QTL region of significant effect on *Salmonella* response traits. Alternatively, whole-genome enabled selection would integrate *Salmonella* QTL into the context of all traits under evaluation and selection in a breeding line. Although selection via gene expression may be more technically challenging, Swaggerty *et al*. (2008, 2009) have proposed and initiated a demonstration of modulating resistance properties to *Salmonella* by means of genetic selection for levels of cytokine expression in meat-type chickens; progeny of sires with high versus low cytokine levels produced progeny with the same relative profile. The work of Beaumont *et al*. (2009), demonstrating a significant negative correlation between resistance to carrier state in young and mature birds, illustrates the importance of clearly defining the breeding objectives to achieve the desired outcomes. Although genetic modulation of the *Salmonella* carrier state can be readily accomplished by divergent selection, the impact of selection may differ extremely between young and mature birds. Improving poultry health by increasing genetic resistance to disease is essential to meet the increasing emphasis of consumers and the poultry industry on animal welfare, food safety, environmental concerns and efficiency of production. Sufficient knowledge exists to begin this improvement now; however, much remains to be learned about the identity of specific, causal genes and their action in resistance mechanisms against *Salmonella*.

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11 *Escherichia coli* and *Salmonella* in Pigs

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Summary

Diarrhoea due to bacterial infections is a problem mainly in the young growing animal, including the pig. Among the bacteria that cause diarrhoea in pigs are various strains of *Escherichia coli* and *Salmonella*. Considerable genetic variation in resistance/susceptibility has been found for both neonatal and post-weaning diarrhoea caused by *E. coli* carrying F4 fimbriae and post-weaning diarrhoea and oedema disease due to *E. coli* strains with F18 fimbriae. The loci for the receptors of both types of fimbriae have been mapped: the F4 receptor(s) to chromosome 13 (SSC13) and the F18 receptor to chromosome 6 (SSC6). Several candidate genes have been suggested for the F4 receptor, among them different mucine genes (*MUC4*, *MUC13*), and a very close association between a single-nucleotide polymorphism (SNP) in an alpha (1, 2) fucosyltransferase gene (*FUT1*) and the F18 receptor has been identified.

Resistance to *Salmonella* infections in mice is associated with the antimicrobial activity of macrophages, and some studies have suggested that it is linked with polymorphism in the *Nramp1* gene. The gene has been identified in several species including the pig, but data are so far lacking concerning association between polymorphism in the porcine gene and resistance–susceptibility to *Salmonella* infection. Using transcriptome profiles, several porcine genes that are differentially up or downregulated during *Salmonella* infection have been identified. Further studies of associations between polymorphisms in these genes and the outcome of *Salmonella* infection may facilitate the development of tools to identify carrier pigs, and lead towards identification of markers that can be used to select for resistant pigs.

Breeding for increased disease resistance can be potentially performed in several ways; excluding susceptible breeding of animals after exposure, marker-assisted selection (MAS) based on closely linked loci or direct selection based on polymorphism in the causative gene. The rapid development in molecular genetics has provided dense genome maps and the tools to identify and study individual genes, both at the deoxyribonuclease acid (DNA) and the expression level. Overall use of genetic markers influencing disease traits is expected to increase significantly in the coming years. This number will grow as large-scale accurate disease phenotypes are collected in pedigreed populations. It is likely that many disease markers will contribute additively to

the selection criteria and will be used as part of complex selection indices that will balance other economically significant traits.

Introduction

Diarrhoea is a common problem in animal production, mostly affecting the young growing animal. A high frequency of all pig litters born are affected with diarrhoea pre-weaning as well as post-weaning, which is responsible for a substantial part of total mortality. Also, piglets that experience diarrhoea may grow more slowly and have poorer performance (Fairbrother and Gyles, 2006).

Within the Swedish pig industry, the routine use of feed additives (antibiotics) was prohibited in 1986. Initially this led to an increased frequency of post-weaning diarrhoea and to decreased productivity of piglets. Thus, specific management and hygienic demands were required to prevent disease outbreaks in Sweden. The total mortality among piglets is today approximately 15% pre-weaning and 2% post-weaning in Swedish conventional herds, but lower in specific-pathogen-free (SPF) herds (N. Lundeheim, Uppsala, 2008, personal communication). These differences and large inter-herd differences obtained between conventional herds demonstrate a large influence of management and environmental factors on health status and productivity of piglets.

During the neonatal period, diarrhoea is generally associated with a small range of pathogens, commonly *Escherichia coli*, *Clostridium perfringens* or *Isospora suis*. In older piglets, various infectious agents may cause diarrhoea, among them bacteria such as *E. coli*, *Salmonella* and *Lawsonia intracellularis*. However, viruses and protozoa can also contribute to the clinical status. Diarrhoea is thus a multifactorial disease where the outcome of an infection is due to multiple factors and their interactions. The presence of various virulence factors, such as fimbriae enabling adherence to intestinal mucosa and enterotoxin production, is essential for the pathogenesis of the bacteria. Although management and housing routines influence the frequency and severity of diarrhoea, the genotype of the pig also has a large impact on the outcome and frequency of clinical infection. This chapter will deal mainly with genetic resistance of pigs to *E. coli* and *Salmonella* infections.

E. coli Diarrhoea

The ability of enteropathogenic *E. coli* (EPEC) or enterotoxigenic *E. coli* (ETEC) strains to adhere to the brush borders of enterocytes is fundamental for the initiation of infection. Attachment of pathogenic bacteria to the mucosa of the small intestine is mediated by fimbrial adhesinos, several of which have been identified both in animal and human EPEC/ETEC strains. In the pig, strains expressing fimbriae of F4 (K88), F5 (K99), F6 (987P) and F41 types dominate during the neonatal period, while strains expressing other types of fimbria such as F18ab (F107), F18ac (2134P) and Av24 are found during the post-weaning period (Fairbrother *et al*., 2005; Nagy and Fekete, 2005).

E. coli F4 infection

A large portion of neonatal diarrhoea is due to infections with *E. coli* strains possessing fimbriae of the F4 type. The frequency of F4 amongst enterotoxigenic strains differs somewhat between countries, but F4 is to date the most prevalent. Three antigenic F4 variants, *ab*, *ac* and *ad*, have been identified, all containing a common *a*-type antigen (Ørskov *et al*., 1964; Guiné and Jansen, 1979). F4ac is the most prevalent and clinically important ETEC variant (Fairbrother *et al*., 2005; Nagy and Fekete, 2005). The three adhesion variants recognize related, but slightly different, carbohydrate structures on host-cell glycoconjugates. Early studies indicated that D-galactoside, *N*-acetylglucosamine, *N*-acetylgalactosamine and D-galactosamine may be involved (Kearns and Gibbons, 1979; Sellwood, 1980), and the F4ac receptor was described as a mucin-type sialoglycoprotein (Erickson *et al*., 1994; Francis *et al*., 1998). More recently, it was suggested that the three F4 variants recognize a core structure and that each variant prefers different modifications to that structure (Grange *et al*., 2002). Galactose has been determined as an essential component in the F4ac recognition of intestinal mucin-type sialoglycoproteins (Grange *et al*., 1998), and terminal β-linked galactose is an essential component of the F4ad adhesion recognition site on neutral intestinal glycosphingolipids (Grange *et al*., 1999). F4ab, but not F4ac and F4ad, binds with high affinity to porcine serum transferrin (Grange *et al*., 2002).

Detection of F4 receptor phenotype pigs

Identification of the receptor phenotype of pigs can be performed *in vitro* by examining the adhesion of *E. coli* F4-positive bacteria to intestinal cell brush borders (Sellwood *et al*., 1975), to whole enterocytes (Rapacz and Hasler-Rapacz, 1986) or to a length of villous brush border (Van den Broeck *et al*., 1999). Mostly, intestinal specimens have been sampled after slaughter, but the technique has also been performed on specimens from intestinal biopsies (Snodgrass *et al*., 1981). An enzyme immunoassay and an enzyme-linked immunosorbent assay (ELISA) have also been developed (Chandler *et al*., 1986; Valpotic *et al*., 1989). Phenotyping of pigs using small intestine mucus has also been suggested as the mucus contains F4-binding glycoproteins and glycolipids (Blomberg *et al*., 1993).

Different cut-off values have been used to differentiate between the adhesive and non-adhesive phenotypes measured by the number of bacteria adhering to brush borders, enterocytes or a length of villous brush border (Bijlsma *et al*., 1982; Hu *et al*., 1993; Edfors-Lilja *et al*., 1995; Baker *et al*., 1997; Python *et al*., 2002, 2005; Jørgensen *et al*., 2003; Li *et al*., 2007; Peng *et al*., 2007; Rasschaert *et al*., 2007; Wang *et al*., 2007a; Zhang *et al*., 2008). Most studies describe an intermediate phenotype with weak adhesion. Depending on the cut-off value used, pigs with this phenotype are scored as adhesive or non-adhesive. The composition of buffers used in the adhesion test differs between studies. Some protocols use an addition of D-mannose to prevent potential binding to *E. coli* Type 1 pili, while other studies use a modified phosphate buffered saline (PBS) without mannose. These discrepancies may

influence the outcome of studies, and may complicate interpretations concerning the genetic basis of resistance.

The F4ab and F4ac adhesive phenotypes have been determined in newborn as well as in adult pigs. In contrast, the weak-adhesion F4ad phenotype could not be detected in pigs after the age of approximately 16 weeks (Hu *et al*., 1993). A similar age influence has also been found for the adhesion of *E. coli* carrying F5 (K99) (Runnels *et al*., 1980) and F6 (987P) fimbriae (Dean-Nystrom, 1995). A variation in the quantity of F4ac receptor along the length of the small intestine has been reported, with the highest level in mucosal scrapings from the mid-small intestine (Chandler *et al*., 1994). Recently, it was shown that isolated F4 fimbriae adhere strongly to the villous epithelium and the follicle-associated epithelium of the jejunum and ileum of F4 receptor-positive ($F4R^+$) pigs (Snoeck *et al*., 2008).

Inheritance of the F4 receptor phenotype

A genetic influence on resistance to ETEC strains was described 40 years ago (Sweeney, 1968), and later dominant inheritance of the F4ac receptor (F4acR) was shown (Sellwood *et al*., 1975; Gibbons *et al*., 1977). However, the number of loci encoding F4abR and F4acR is still not certain. A one-locus model (Rapacz and Hasler-Rapacz, 1986; Bijlsma and Bouw, 1987; Python *et al*., 2002; 2005; Jørgensen *et al*., 2003; Joller *et al*., 2006) has been suggested, while other studies indicate two closely linked loci (Guérin *et al*., 1993; Edfors-Lilja *et al*., 1995; Li *et al*., 2007; Peng *et al*., 2007; Wang *et al*., 2007a; Zhang *et al*., 2008). The inheritance of F4adR has been less studied, but dominant inheritance with incomplete penetrance has been suggested (Hu *et al*., 1993). A separate locus for F4adR (Bijlsma and Bouw, 1987), as well as one common locus for F4abR/acR/adR (Hu *et al*., 1993), has been proposed.

In the context of a two-locus model, strong linkage disequilibrium between the two loci has been found in several breeds studied (Bijlsma *et al*., 1982; Edfors-Lilja *et al*., 1986, 1995; Rapacz and Hasler-Rapacz, 1986; Hu *et al*., 1993; Baker *et al*., 1997; Python *et al*., 2005; Li *et al*., 2007), with most pigs (90% or more) positive or negative for both F4abR and F4acR. However, a somewhat higher frequency of the $F4abR^+F4acR^-$ phenotype was found in the Hampshire breed, i.e. 4 of 24 tested pigs (Baker *et al*., 1997). A higher frequency of the $F4abR^-F4acR^+$ phenotype was found in a White Duroc × Erhualian inter-cross, namely 156 of 640 tested F2 animals (Wang *et al*., 2007a). Strong linkage disequilibrium would be expected if haplotypes either positive or negative for both F4abR and F4acR are favoured by selection. Other causes are if there has not been sufficient time since the initial mutation for recombination to occur, or that linkage disequilibrium has remained by chance in small populations. The recombination frequency may also be overestimated, in studies that suggest two loci, as a result of typing errors or incomplete penetrance. Differences in the adhesion test methodology to evaluate the phenotype, as described above (type of tissue, cut-off value used to differentiate between phenotypes, adhesion test buffer composition), may account for the different results.

Chromosomal localization and candidate genes

Testing potential breeding animals using intestinal biopsies or test mating is costly and cumbersome, nor can any of the described assays differentiate between pigs carrying one or two copies of the receptor allele, i.e. distinguish between heterozygous and homozygous animals. Identification of the gene coding for the receptor structure potentially makes direct typing of breeding animals possible, and would facilitate genotype identification for individual animals.

The F4ac receptor locus is linked to the transferrin locus on SSC13 (Gibbons *et al*., 1977; Guérin *et al*., 1993; Edfors-Lilja *et al*., 1995), and the locus has been fine-mapped close to microsatellites Sw207 and S0075 in the SSC13q41 band (Python *et al*., 2002, 2005; Jørgensen *et al*., 2003). Refined linkage mapping shows that the F4acR locus is located between S0283 and S0075 as the most probable position (Joller *et al*., 2006; D. Joller, Zürich, 2008, personal communication). This chromosomal region is homologous to human chromosome 3 and, using comparative mapping, several candidate genes have been identified and examined. Significant linkage disequilibrium has been described between the F4abR and/or F4acR loci and single nucleotide polymorphisms (SNPs) in the mucin genes *MUC4* (Jørgensen *et al*., 2004; Joller *et al*., 2006; Peng *et al*., 2007) and *MUC13* (Zhang *et al*., 2008), and in the transferrin receptor gene (Wang *et al*., 2007a). Screening of 24 genes in the candidate region revealed 177 SNPs in 12 of the genes and a large conserved haplotype block around *MUC4* on the F4ab/F4ac susceptible chromosomes, suggesting that the causative mutation(s) has (have) occurred quite recently (Jacobsen *et al*., 2009).

Selection for the F4 receptor phenotype

The newborn pig is dependent on the mothering capacity of the sow, including antibodies and immune cells provided by colostrum and milk. Sows lacking the receptor produce low levels of antibodies to F4 after natural exposure or oral vaccination/immunization (Sellwood 1979, 1982; Bijlsma *et al*., 1987; Verdonck *et al*., 2004). The highest risk for diarrhoea will thus be for susceptible piglets born to sows that lack the receptor. A small, but significantly higher, immunoglobulin G (IgG) response has been found in F4abR$^+$/F4acR$^+$ pigs 3 weeks after an intramuscular immunization, suggesting that the immunization acted as a booster dose in receptor-positive pigs (Edfors-Lilja *et al*., 1995).

Although the receptors mediate increased susceptibility to neonatal *E. coli* diarrhoea, the function and significance of the F4 receptor(s) at a more basic level is not known. A low frequency of pigs possessing the receptor(s) has been identified in breeds not selected for increased growth. No F4acR$^+$ phenotype pigs were identified in the Chinese Meishan breed (Chappuis *et al*., 1984; Michaels *et al*., 1994; Rita *et al*., 1994), and only a low frequency of the F4acR$^+$ phenotype was found in the Chinese Minzu breed (Michaels *et al*., 1994; Rita *et al*., 1994). Also, the Songialo Black breed, composed of Minzu, Landrace and Duroc, has a low frequency of the F4acR$^+$ phenotype (Li *et al*., 2007). A weak adhesion to intestinal cells, but with no correlation to virulence, was found for Chinese Meishan pigs (Bertin and Duchet-Suchaux, 1991).

Both European wild boars that were used as parents in a reference pedigree for gene mapping were F4abR$^-$/acR$^-$ (Edfors-Lilja *et al*., 1995).

SNPs in *MUC4* show significant linkage disequilibrium with the *F4abR* and *F4acR* loci and a DNA marker test based on one of these SNPs has been developed (Jørgensen *et al*., 2004). Seventy-four per cent of young pigs carrying the allele associated with susceptibility had diarrhoea 1 day after oral challenge with ETEC F4ac, compared with 20% of the pigs lacking this allele (Jensen *et al*., 2006).

E. coli F18 infection

Diarrhoea in the older pig is often associated with strains of *E. coli* other than those causing neonatal diarrhoea. The change in diet at weaning and some nutritional components are thought to predispose to diarrhoea and/or oedema disease. Oedema disease is caused by an exotoxin produced by strains of *E. coli* F18 that damages vessel walls in multiple tissues such as brain, stomach, intestine and lungs, resulting in significant morbidity and mortality losses. The frequency of these problems differs largely between countries and populations, but breed differences have also been observed. Oedema disease and post-weaning diarrhoea are responsible for considerable economic losses and can be a problem even in adult pigs (Fairbrother *et al*., 2005), and the occurrence of F18 ETEC strains is high in several countries, e.g. Belgium (Verdonck *et al*., 2003) and the USA (Zhang *et al*., 2007).

The ETEC strains that cause post-weaning diarrhoea and oedema disease mostly colonize the small intestine by means of F4 or F18 fimbriae (Imberechts *et al*., 1992). Like the F4 fimbria, the F18 fimbria possesses a common antigenic variant, *a*, and two variant-specific determinants, *b* and *c* (Rippinger *et al*., 1995).

Detection and inheritance of the F18 receptor phenotype, chromosomal localization and candidate genes

As with neonatal diarrhoea, a genetic influence on the frequency of post-weaning diarrhoea and oedema disease was described 40 years ago (Smith and Halls, 1968). After the development of the adherence assay for identification of the F4R phenotype, similar studies were performed to identify pigs resistant to *E. coli* F18 infection. These studies showed that susceptibility to colonization by F18ab$^+$ *E. coli* is dominantly inherited (Bertschinger *et al*., 1993). Further studies mapped the locus for the F18abR to SSC6, close to the genes for blood group system S and the 'halothane' locus (ryanodine receptor (RYR)) (Vögeli *et al*., 1996). The *F18R* locus was later linked to two alpha (1, 2) fucosyltransferase genes (*FUT1* and *FUT2*) that code for enzymes catalysing production of blood group antigen structures (Meijerink *et al*., 1997). An SNP in *FUT1*, G or A at nucleotide 307 co-segregates with *E. coli* F18 adhesion (or lack of adhesion) where the recessive $FUT1^A$ allele confers resistance. A polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) test based on this polymorphism, regarded as the causative mutation, has been developed (Meijerink *et al*., 2000).

Selection for the F18 receptor phenotype

Alpha (1, 2) fucosyltransferases are essential in the formation of the porcine AO blood group antigens, in correspondence with the human ABO blood group system. They transfer a fucose to H precursors, leading to the synthesis of the H blood group antigens. Expression of the A blood group antigen is due to another enzyme, but the A blood group phenotype is dependent on the expression of its precursor, the H antigen. It has been shown that $FUT1^A$ homozygous pigs have decreased levels of FUT1 activity (Meijerink *et al*., 2000) and a very low expression of blood group antigens on their small intestinal epithelial cells (Coddens *et al*., 2007). A majority of $FUT1^A$ homozygous pigs showed no H or A antigen expression and no *E. coli* F18 adherence (Coddens *et al*., 2007). Experimental challenge with an $F18^+$ *E. coli* strain showed that most (10/14) of the genetically susceptible $FUT1^{GA}$ and $FUT1^{GG}$ pigs developed diarrhoea, but only 1 of 17 resistant $FUT1^{AA}$ pigs did (Frydendahl *et al*., 2003).

The frequency of the F18-resistant $FUT1^A$ allele has been estimated to be approximately 20% in the Belgian (Coddens *et al*., 2008) and Swiss (Meijerink *et al*., 1997) pig populations, as well as in Landrace and Duroc breeds in Taiwan (Huang *et al*., 2008). As mentioned previously, *FUT1* is mapped to the 'halothane' linkage group on SSC6, where the $RYR1^T$ allele confers susceptibility to malignant hyperthermia (Fujii *et al*., 1991). No linkage disequilibrium was found between the *FUT1* and *RYR1* loci in the Belgium pig population (Coddens *et al*., 2008), in contrast to what was previously found for Swiss Landrace with a 93% association between $FUT1^A$ and $RYR1^T$ (Meijerink *et al*., 1997). In Landrace and Duroc breeds in Taiwan, no association was found between *FUT1* genotype and growth performance traits (Huang *et al*., 2008). In conclusion, the possibility of combined selection for both F18- and stress-resistant pigs depends on the pig population and its history.

Salmonella Infections

Salmonella enterica infections are an important human health problem in many countries, and swine, poultry, cattle and seafood can be important carriers (Thorns, 2000). Various strategies are used to reduce the risk to humans of contracting salmonellosis from animal-derived products, including pork. In Sweden, an official control programme with respect to *Salmonella* spp. was initiated in 1961 (Anon., 1995). Regular controls are performed at the slaughterhouses and also the feed plants. Regardless of the source, all *Salmonella* isolations have to be reported (Swedish Zoonosis Act; SFS, 1999, p. 658). Infected farms are subjected to extensive restrictions including a total ban of movements of animals, with the exception of transportation for sanitary slaughter. As a service to farmers, a *Salmonella* health control programme was initiated in 2002 by the Swedish Animal Health Service. Together these measures have resulted in very low prevalence of positive animals, and only a few farms with positive animals are detected per year (Anon., 2005). In Denmark, a control programme was initiated in 1993 in response to increasing numbers of human cases of salmonellosis arising from pork consumption

(Mousing *et al*., 1997). The programme is based on serological sampling of all herds that produce more than 200 pigs per year. Farms are classified according to a serological *Salmonella* index with three levels. Farms on levels two and three are subjected to lower carcass payments and undergo on-farm investigations. The programme has been effective and the prevalence of *Salmonella* monitored at slaughterhouses has been reduced to less than 1%. Salmonellosis in humans due to pork consumption decreased from 22 to 3 cases/100,000 from 1993 to 2001 (Wegener *et al*., 2003). To improve cost-effectiveness, a risk-based surveillance approach is now being discussed (Benschop *et al*., 2008). In addition, the EU has established legislation to make *Salmonella* monitoring and control programmes mandatory for its member countries (EC, 2160/2003) in an effort to minimize zoonotic infections.

Salmonella spp. in pigs

The most frequently reported *Salmonella*, *S. enterica* serovar *Typhimurium*, has a broad host range including humans and food-producing animals, while *S. enterica* serovar *Cholerasuis* is regarded as host-restricted to pigs, although it may cause disease in a limited number of other species. Clinical salmonellosis in pigs is mostly caused by these two serovars (Fedorka-Cray *et al*., 2000). *S. cholerasuis* causes a systemic infection, often including respiratory signs and high mortality. Infection with *S. typhimurium* mostly causes watery diarrhoea, has a low mortality rate and no systemic effects. As the infection may become persistent, with asymptomatic excretion of bacteria for several months after exposure, *S. typhimurium* is a major pathogen in pork-related human salmonellosis. During 1999–2000, this serovar was detected in approximately 10% of slaughter pigs in the UK (Davies *et al*., 2004). *S. cholerasuis* was once the most frequently isolated serovar in pigs in the UK but is now rarely found, while it is more common in the USA, as reviewed by Paulin *et al*., (2007).

Following oral infection, *S. typhimurium* replicates fast in the intestinal cell wall and induces rapid pro-inflammatory responses, including those involving TNF-A, IL-8 and IL-18, whereas *S. cholerasuis* replicates slowly, induces fewer pro-inflammatory responses and these are associated with enhanced persistence in the mesenteric lymph nodes (Paulin *et al*., 2007). Using a jejunal epithelial cell line, Skjolaas *et al*. (2006) showed that serovars *S. typhimurium* and *S. cholerasuis* induce a different upregulation of cytokines and chemokines important for movement of macrophages (MIF), neutrophils (IL-8), dendritic cells (CCL20) and epithelial remodelling (osteopontin (OPN)).

Feed materials are a potential source of spread of *Salmonella* infection in pigs, including several different *Salmonella* serovars (e.g. Derby, Cubana and Yoruba). However, experimental challenge with *Salmonella* has mostly focused on *S. typhimurium* and *S. cholerasuis*, and many of the serovars detected in feed material are poorly documented, as are pigs' immune responses against them. In a recent challenge study, it was shown that the response to *S.* Yoruba was slower and of a lower magnitude than the response to *S. typhimurium* (Österberg and Wallgren, 2008).

Genetic resistance and candidate genes

Prevalence of salmonellosis can be reduced substantially by non-genetic actions, but control programmes are expensive and increased genetic resistance would be beneficial. In mice, a high level of resistance to *S. typhimurium* infection and other facultative intracellular bacteria is determined by the *Ity/Lsh/Bcg* gene (Skamene *et al*., 1982), later named natural resistance-associated macrophage protein gene (*Nramp1*(*Slc11A1*)) (Vidal *et al*., 1995). Nramp1 is a member of a family of membrane-transport proteins identified in many species including humans, chickens, *Drosophila*, and various plants and yeasts (Cellier *et al*., 1995; 2007). Nramp1 expression is localized to late endosomes/lysosomes where it delivers divalent cations from the cytosol to phagolysosomes and plays an important role in macrophage activation in infectious and autoimmune disease.

Association between *Nramp* polymorphism and resistance/susceptibility to *Salmonella* infection is well documented in mice. Mice with a mutant Nramp1 allele have a bias towards a T helper 2 response and are susceptible to *S. typhimurium* infection. In humans, polymorphism in the *Nramp1* promotor region is associated with resistance/susceptibility to tuberculosis, leprosy and the autoimmune diseases rheumatoid arthritis, Crohn's disease and diabetes (for reviews see Blackwell *et al*., 2000; Gruenheid and Gros, 2000). *Salmonella* resistance in chickens is covered elsewhere in this book.

The *Nramp1* gene has been identified and chromosomally assigned also in the pig. Two slightly different cDNA sequences are published for porcine *Nramp1* (Tuggle *et al*., 1997; Zhang *et al*., 2000). The gene is assigned to SSC15, and a small population study revealed large allele frequency differences among breeds (Sun *et al.,* 1998). Associations between *Salmonella* bacterial counts and *Nramp1* polymorphisms are currently being explored (C. Tuggle, Iowa, 2008, personal communication), but so far no results have been published.

Toll-like receptors (TLRs) recognize pathogen-associated molecular patterns and play a critical role in activation of the immune system. Genes corresponding to all ten *TLRs* identified in humans have been cloned in pigs (reviewed by Uenishi and Shinkai, 2008). TLR2 participates in the recognition of Gram-positive bacteria (peptidoglycans, lipoteichoic acid), TLR4 recognizes Gram-negative bacteria (LPS, lipid-A), TLR5 recognizes flagellin and TLR9 recognizes unmethylated CpG DNA, and these TLRs are all expressed in the intestine and mesenteric lymph nodes. Both TLR5 and TLR9 are upregulated in the pig after challenge with *S. typhimurium* and *S. cholerasuis* (Burkey *et al*., 2007), but no associations with resistance/susceptibility have been found so far.

To map resistance genes for *S. cholerasuis*, a pig reference family was bred (van Diemen *et al*., 2002). After oral challenge, significant differences were found between sire families. Survival rate was variable and the most resistant piglets had higher numbers and better function of polymorphonuclear neutrophils, but lower lymphocyte mitogen response and lower antibody response to LPS from *Salmonella* spp. Using data from this and other populations, several chromosomal regions have now been identified and associated with

Salmonella resistance through associations with bacterial counts in organ tissues as a measure of resistance (Galina-Pantoja *et al*., 2009).

The transcriptional response to *S. enterica* has recently been explored in a series of studies. Differential gene expression in mesenteric lymph nodes after infection with *S. typhimurium* or *S. cholerasuis* revealed significant differences in expression patterns (Uthe *et al*., 2006, 2007; Wang *et al*., 2007b). *S. typhimurium* induced expression of genes involved in T helper 1, innate/inflammatory and antigen-processing pathways 24 h post-infection, while apoptosis and antigen presentation/dendritic cell function pathways were downregulated at 8 h post-infection. *S. cholerasuis* induced significantly higher levels of gene expression at later time points, 48 h to 21 days. Both microarray and real-time quantitative PCR data provided evidence of a strong NFκ-B-dependent transcriptional response during *S. cholerasuis* infection (Wang *et al*., 2008). Gene expression profiling has also been performed in lung tissue after *S. cholerasuis* infection, showing differential expression of many genes (Zhao *et al*., 2006). Transcriptional profiling has the potential to identify candidate genes for selection; e.g. an association was found between an SNP in *CCT7*, one of the upregulated genes following *S. typhimurium* challenge, and *Salmonella* shedding 7 days post-infection (Uthe *et al*., 2009).

Use of Disease Markers in Animal Breeding

The use of multiple genetic markers as part of livestock breeding programmes is fairly new. Nowadays, marker genotype information is being added routinely in pig genetic improvement programmes as part of complex quantitative breeding schemes that balance multiple selection objectives. The implementation of specific genetic markers depends on a number of factors such as size of the marker effect, its mode of inheritance and pleiotropic effects on all economically significant traits, and not just the trait or traits targeted in discovery.

Initially marker usage was limited to single genes with major effects. In 1991, 'halothane' gene testing became available to eliminate the recessive RYR^T allele associated with porcine stress syndrome and pale, soft and exudative pork (Fujii *et al*., 1991). Later on, other single-gene tests also became available, including the RN (Rendement Napole) 'acid meat' gene test (Enfält *et al*., 1997) and a DNA marker test for increased litter size (Rothschild *et al*., 1997).

The use of a DNA marker test based on an intronic SNP in the *MUC4* gene (Jørgensen *et al*., 2004) started in 2003 in a commercial breeding programme (Danish Pig Production annual report, 2004) to increase resistance to diarrhoea caused by F4 *E. coli*. Danbred has used the marker alongside the usual selection index in their Landrace, Large White and Duroc lines. The selection strategy involved identifying homozygote resistant or carrier boars at the nucleus level and more recently in multiplication herds. The current strategy is to genotype 100% of boars and only a limited number of sows (Danish Pig Production annual report, 2007). The frequency of *MUC4* genotypes linked to resistance is estimated to have increased for Landrace boars from 1% to 11% by 2005, with an additional 54% being carriers (Danish Pig Production

annual report, 2006). Resistant genotypes for Large White and Duroc boars are approaching 100% frequency from an original 20% and 90%, respectively (Danish Pig Production annual reports, 2006 and 2007).

A DNA marker in the *FUT1* gene became part of a commercial breeding programme in 2005 to select for resistance to oedema disease and post-weaning diarrhoea caused by F18 *E. coli* (PIC, unpublished information). The DNA marker is used to increase frequencies of the favourable $FUT1^A$ allele independently of index selection in the lines. Maternal Landrace, Large White and Duroc lines have been selected using this strategy and frequencies have improved from 0.17, 0.53 and 0.30 in 2005 to 0.43, 0.7 and 0.69 in 2008, respectively (PIC, unpublished information). Studies of weaned piglets that were challenged with $F18^+$ *E. coli* showed a reduction in death loss from 22% to less than 1% in pigs with two copies of the $FUT1^A$ resistance allele (Mellencamp *et al*., 2003). In surviving pigs, resistant pigs demonstrated a 30% improvement in average daily weight gain over susceptible pigs (Mellencamp *et al*., 2003). In addition, resistance at the population level may not require all pigs to be homozygous for the *FUT1* resistant allele, but rather just a proportion of the animals. This proportion will depend on the basic reproductive ratio or the transmission rate for the disease (Bishop and MacKenzie, 2003).

Resistance to most diseases is, however, multifactorial. Mallard and Wilkie (2007) suggested there were probably about 2000 genes involved in disease resistance in the pig. In the pig, resistance to pathogens such as *Salmonella* and porcine reproductive and respiratory syndrome virus (PRRSV) probably falls in this category (Roy and Malo, 2002; Galina-Pantoja *et al*., 2006). Integration of QTL mapping and transcriptional profiling of the same population will improve our understanding of the genetic background to complex traits (Tuggle *et al*., 2007). Implementation of marker technology in disease resistance is more complex when many unknown genes are involved. *Salmonella*, for instance, targets many pathways and there are likely to be many genes involved in conferring resistance that contribute quantitatively to overall resistance. Although several chromosomal regions have been identified and associated with *Salmonella* resistance as described above (Galina-Pantoja *et al*., 2009), further validation is required before implementing these markers in commercial breeding programmes.

A key requirement in further discovering and validating multifactorial disease markers is databases that have phenotypes representative of disease traits, along with DNA from the phenotyped animals. Unfortunately such datasets are very limited. Traditionally efforts have been directed at providing disease-specific phenotypes through challenge models. However, challenge datasets usually lack sufficient numbers for case-control marker discovery, which often requires thousands of animals for sufficient statistical power. More recently, as marker technology has moved to more powerful and affordable platforms, commercial breeding companies have increased efforts to collect disease phenotypes representative of traits of economic importance under field conditions. Currently the focus is on traits associated with robustness such as pre-weaning mortality, nursery mortality, grow–finish mortality, culls, lightweight pigs and growth under commercial conditions. In addition, genomic technology has advanced from the economic need to identify

markers in candidate genes to using whole-genome scans utilizing the tens of thousands of SNPs created from sequencing projects. As a result, numerous markers associated with a larger number of traits are being found, and the challenge and opportunity for the breeding industry lies in implementation of these markers into an integrated quantitative breeding programme.

After marker associations are discovered, there follows a rigorous validation process to determine their impact in multiple lines and across multiple traits in the breeding programme. Such testing is required before markers are implemented in line development. An economically and technically balanced approach to the use of disease resistance or robustness markers is the key to a successful implementation. DNA marker profiles are only one component of breeding programme information systems. Other components include phenotypic performance data measured at the genetic nucleus and in cross-bred pigs under commercial conditions. Intensive selection on health traits would decrease the pressure on other economically important traits, and would be justified only when the heritability and/or variability is large and the economic benefit outweighs the opportunity cost of reducing selection for other traits in the selection indexes.

Overall use of genetic markers influencing disease traits is expected to increase significantly in the coming years. This number can grow as large-scale accurate disease phenotypes are collected in pedigreed populations and as high-density genome scans are applied to multifactorial robustness and mortality traits of unknown specific aetiology but representative of relevant commercial environments. Many such markers will increasingly contribute to the accuracy of estimated breeding values used in indices designed to improve overall profitability of pork production.

Conclusions

Diarrhoea due to bacterial infections is a problem in pig production, both with regard to loss in productivity and also from a human health view, as the pig can act as a carrier. Genetic variation in resistance to neonatal diarrhoea caused by *E. coli* carrying F4 fimbriae has been known for many years. Also, genetic variation in resistance to post-weaning diarrhoea and oedema disease due to *E. coli* with F18 fimbriae has been identified. Loci for both types of receptors have been mapped, although questions still remain about the nature of the F4R locus. Candidate/closely linked genes have been identified for both the F4R and F18R loci, and DNA-based tests have been developed to enable the genotyping of breeding animals. These tests are currently used by commercial breeding companies to increase resistance in their pig populations.

Resistance to *Salmonella* infections is not associated with any known receptor molecule, but resistance is associated with the innate immune response. In mice, this resistance is associated with polymorphism in the *Nramp1* gene. This gene has been identified also in the pig, but data are still sparse for associations with resistance/susceptibility to *Salmonella* infection. Ongoing studies are focusing on differential gene expression during infection to identify potential markers for resistance.

The rapid development of molecular genetics has given us dense genome maps and the tools to identify and study individual genes. This will increase the potential to select breeding animals of preferred genotype. How to decide which genotype to select? This requires extensive datasets comprising phenotypic databases plus DNA samples, and the ability to estimate the pleiotropic effects on economically significant traits. In addition, knowing the function and biological impact of the genes in question should be beneficial in ways other from selection. Some data suggest that pig populations not selected for growth have a low frequency of the F4 receptor, but we do not have sufficient results to determine whether receptor phenotype pigs grow faster. Even fewer studies concerning the influence of the F18 receptor on production traits have been reported. As regards *Salmonella* infections, it has been suggested that increasing resistance might increase the frequency of autoimmune diseases.

In conclusion, we know far more about genetic variation in resistance to bacteria causing diarrhoea than we did in the recent past. The development of DNA-based tests for resistance to *E. coli* enables us to determine the resistance genotype of individual animals, and hence the use of these tests already allows breeding for increased resistance to the major types of *E. coli* infection. In addition, the ability now to conduct genome-wide scans with tens of thousands of DNA markers makes it possible to identify markers for production traits and all relevant diseases. The challenge will remain, however, of having large databases with phenotypic data relevant to diseases available for use. In the near future it is expected that disease markers will contribute additively to selection criteria and will be used as part of selection indices that will balance other economically significant traits.

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12 Genetic Aspects of Resistance to Ovine Footrot

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Summary

Footrot of sheep is an infectious disease resulting from invasion of epidermal tissue of the hoof by a mixed group of bacteria, particularly *Dichelobacter nodosus*, a Gram-negative anaerobic bacterium. The disease may progress to separation of the sole, soft and hard horn from the underlying hoof matrix. In severe cases, footrot is a debilitating disease associated with acute lameness, it impacts on animal welfare and it causes substantial economic losses. Footrot has been reported in almost all sheep-raising countries around the world. Under field conditions, footrot severity may be conveniently scored on a scale where 0 indicates normal feet, 1 is limited interdigital dermatitis or scald, and 2–4 indicate increasing severity of footrot. Resistance, assessed either on single or repeated scores, or on presence or absence of footrot, has been shown to be a heritable trait in a variety of breeds under contrasting environmental conditions, and for both deliberate and natural challenge. Such heritabilities are generally close to 0.20, with the heritability of liability to footrot being slightly higher. Further, significant additive genetic variation has been demonstrated in Merino sheep for response to *D. nodosus* vaccines. Genetic markers for resistance would be beneficial; however, strong and consistent associations would be required to justify their application in the field. Currently there is sufficient preliminary information available on relevant genetic parameters for footrot resistance, as well as its correlations with other production traits, to include resistance to footrot in sheep-breeding programmes. However, it is not sensible to consider selection for footrot resistance without also considering non-genetic disease control. An overall programme to improve animal health and performance will need to consider all economically feasible forms of disease control, along with optimized selection strategies.

Introduction: Footrot in Sheep

A detailed account of all aspects related to footrot is beyond the scope of this chapter. There is an excellent overview of footrot in ruminants by Egerton *et al*. (1989), and in particular a comprehensive and detailed chapter on footrot of

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sheep is given by Stewart (1989). For those with a specific interest in footrot, the benchmark publication is that of Beveridge (1941), and reviews by Allworth (1995) and Abbott and Lewis (2005) also provide an excellent overview of the subject. Egerton (2008a) provides an excellent historical account of the development of our understanding of footrot and relevant control methods.

Definition of footrot

Footrot of sheep is an infectious and, on specific occasions, an exceptionally contagious disease resulting from invasion of epidermal tissue of the hooves by a mixed group of bacteria (Egerton *et al*., 1969; Roberts and Egerton, 1969). An essential component of this mixture is *Dichelobacter nodosus*, formerly *Fusiformis nodosus* (Beveridge, 1941) or *Bacteroides nodosus* (Dewhirst *et al*., 1990; Moore *et al*., 2005a). *D. nodosus* is a Gram-negative anaerobic bacterium which, so far as is known, occurs naturally only in the feet of ruminants affected by footrot. The disease is characterized by infection of the interdigital skin (IDS), and may under certain conditions progress to separation of the sole, soft and hard horn from the underlying hoof matrix. In severe cases footrot is a debilitating disease associated with acute lameness, and it impacts on animal welfare and economic losses associated with lost production and control, therapeutic and preventative measures (Grogono-Thomas and Johnston, 1997; Winter, 2008). Footrot has been reported from almost all sheep-raising countries around the world.

Foot conditions not classified as footrot

Stewart (1989) provided a comprehensive description of differential diagnosis of foot conditions and diseases associated with lameness in sheep. The most common infections/conditions include ovine interdigital dermatitis (OID), a necrotizing infection by *F. necrophorum*, or scald in the absence of *D. nodosus* (Parsonson *et al*., 1967). Significant confusion still exists about differentiation of OID since the condition may be scald, benign footrot or early stages of virulent footrot. Infection of the IDS with treponemes, an emerging contagious ovine interdigital dermatitis (CODD) (Wassink *et al*., 2003; Moore *et al*., 2005b), gives further rise to incorrect classification of foot lesions associated or diagnosed as footrot. Other lameness can be attributed to foot and toe abscess, and 'shelly toe', although experienced clinicians and stockmen can readily distinguish these conditions from footrot. Less common conditions/infections of feet include strawberry footrot, post-dipping lameness, toxic laminitis, scabby mouth, ulcerative dermatosis, bluetongue and foot-and-mouth disease. None of these conditions is associated with *D. nodosus* and, with the exception of OID, experienced clinical examiners can readily distinguish these conditions from ovine footrot. Although at farm level, those associated with regular care of footrot can recognize the condition

as footrot, the terminology to describe the condition is often used incorrectly (Kaler and Green, 2007).

Summary of the aetiology and pathogenesis of footrot

Infection of normal, dry, healthy IDS of sheep with *D. nodosus* alone is insufficient for the development of footrot. Predisposition of feet through water maceration and activity of normal environmental skin microflora, including *Corynebacterium pyogenes* and various surface-located diphtheroid bacteria, is an essential prerequisite for the development of footrot. Footrot is therefore a mixed bacterial infection, and *Fusiformis necrophorum*, a faecal organism, is essential to the disease. *F. necrophorum* is often considered the main pathogen in the predisposition and early stages of initiation of footrot. Predisposition causes sufficient inflammation and damage to the *stratum corneum* for infection to proceed. The synergistic relationship between *F. necrophorum* and *D. nodosus* leads to progressive and destructive infection of the epidermis of the hoof causing, in severe cases, a separation of the horn from the dermis. The underlying dermis may become inflamed but is not invaded, and the *stratum germinativum* is not destroyed. The extent of tissue damage is dependent on bacterial and host-resistance factors described below.

Transmission of the disease is facilitated directly by transfer of infected material containing *D. nodosus* from exposed lesions into the environment, thereby contaminating the feet of other sheep. Successful transmission is only possible under wet and warm conditions, in sheep that have been sufficiently predisposed. *D. nodosus*, as the obligate parasite, is thus considered the essential transmitting agent of footrot, but development of footrot requires the symbiotic relationship with *F. necrophorum*, as a normal environmental inhabitant, and the action of prolonged wetting of feet.

Expression of Footrot

Clinical signs in individual sheep

Following infection with *D. nodosus* and in the successful development of footrot, a range of clinical signs may be evident. Inflammation, characterized by diffuse superficial necrosis and erythema of the IDS, is evident during predisposition and initial onset of footrot. In more severe cases, a break at the skin–horn junction is visible approximately a week after infection. Extensive separation, which commences at the heel and the posterior region of the sole, may progress along the sole to the toe. In extremely severe cases, separation will extend to the abaxial wall of the hoof.

Chronic infection will cause the horn to be overgrown and misshapen, with extensive necrotizing damage to underlying soft tissues. Apparent self-cure is possible under dry conditions, particularly in cases where the infection has been confined to the IDS.

Scoring of lesions

In order to standardize the description of severity and progression of lesions, a number of scoring systems have been developed for the subjective assessment of footrot. Egerton and Roberts (1971) were the first to propose a scoring system for footrot lesions as follows:

Score 0: normal dry or wet foot.
Score 1: limited interdigital dermatitis.
Score 2: more extensive interdigital dermatitis.
Score 3: severe interdigital dermatitis and under-running (separation) of the horn of the heel and sole.
Score 4: as for 3, but with under-running extended to the walls of the hoof.

Other scoring systems have evolved from this system in an attempt to differentiate more between various levels of progression of footrot infection. An example of a clinical scoring system for use in the New South Wales sheep industry is shown in Fig. 12.1. Raadsma *et al*. (1991) and Conington *et al*. (2008a) have successfully used this scoring and classification system as the basis for describing differences between animals in relative susceptibility.

The prevalence of sheep affected with footrot in different environments exposed to agents of varying virulence can range from negligible (<5%) to extremely high (>95%). This was illustrated by Egerton and Raadsma (1991). For convenience, footrot at the flock level can be classified as described by Egerton and Raadsma (1991).

SEVERE FOOTROT. High proportion (>10%) of animals with severe lesions (score 3 or score 4); rapid development; severe production losses; little evidence of self-cure. This is a severe and debilitating disease with significant lameness and welfare implications.

MODERATE FOOTROT. Disease expressed at a severity between severe and mild footrot. A low proportion of sheep may express severe lesions that are usually confined to under-running of the sole of the hoof (score 3); self-cure is evident; few sheep remain chronically infected. Fewer sheep show lameness, although the few sheep with severe lesions may show acute lameness, and may remain chronically infected.

MILD FOOTROT. Very low proportion (<1%) of animals with severe lesions, with most lesions confined to the IDS (score 1 or score 2). Most lesions resolve spontaneously with onset of dry conditions; little effect on production; associated lameness is less than in severe or moderate footrot. Expression of mild footrot is further complicated in that it closely resembles OID.

It should be noted that these categories are subjective, and a wide range of expression of footrot is usually recognized in the field. The expression of footrot based on the severity and economic impact of the disease in a flock is, in fact, a continuum.

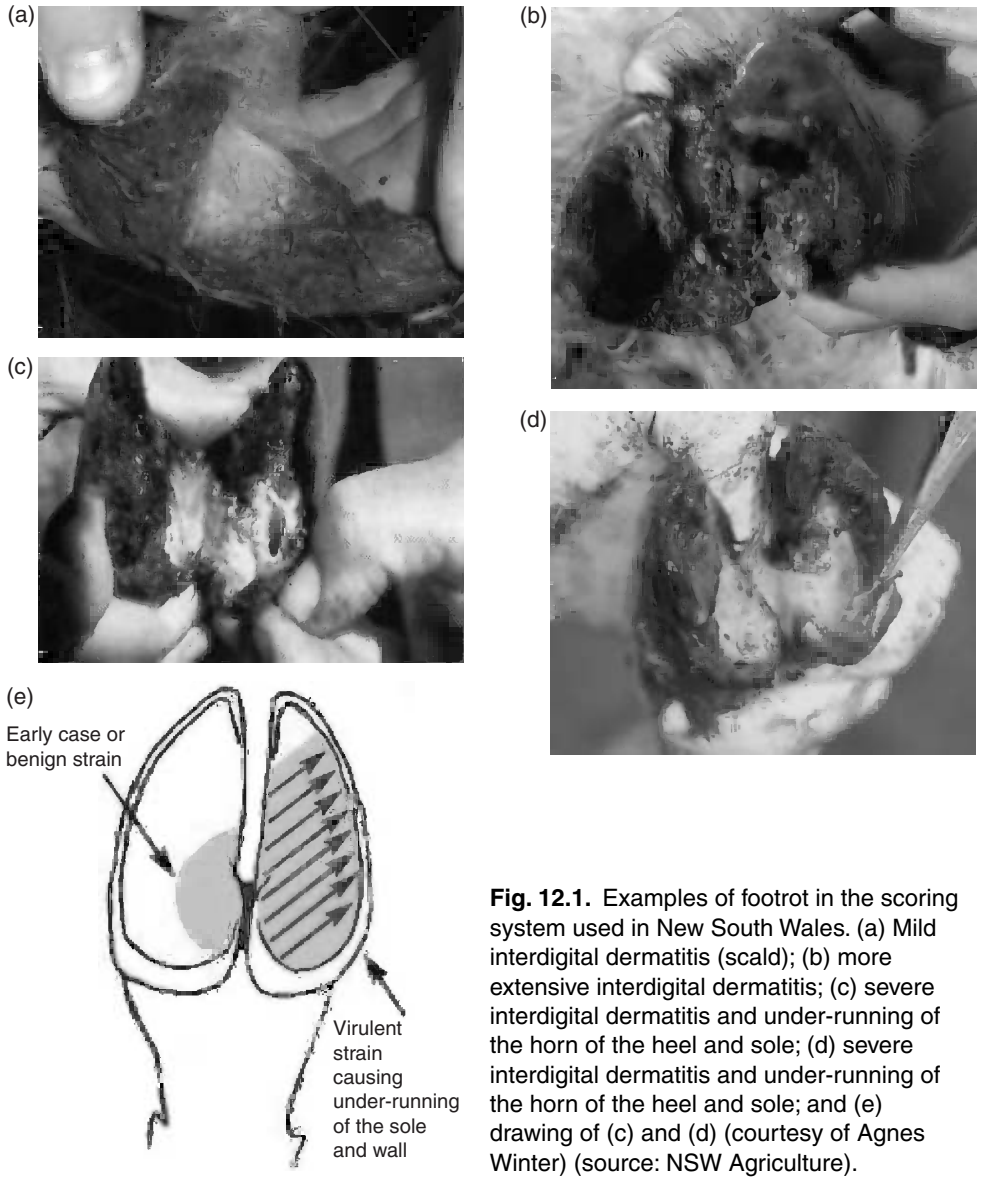

Fig. 12.1. Examples of footrot in the scoring system used in New South Wales. (a) Mild interdigital dermatitis (scald); (b) more extensive interdigital dermatitis; (c) severe interdigital dermatitis and under-running of the horn of the heel and sole; (d) severe interdigital dermatitis and under-running of the horn of the heel and sole; and (e) drawing of (c) and (d) (courtesy of Agnes Winter) (source: NSW Agriculture).

Factors affecting expression of footrot

The expression of footrot in a flock of sheep is governed by three factors: (i) virulence of *D. nodosus*; (ii) suitability of the environment for predisposition of the host and transmission of the organism; and (iii) inherent susceptibility of the host. These factors have been described in detail by Egerton and Raadsma (1991). Their influence on the expression of the disease is shown in Fig. 12.2. It should be recognized that control of the

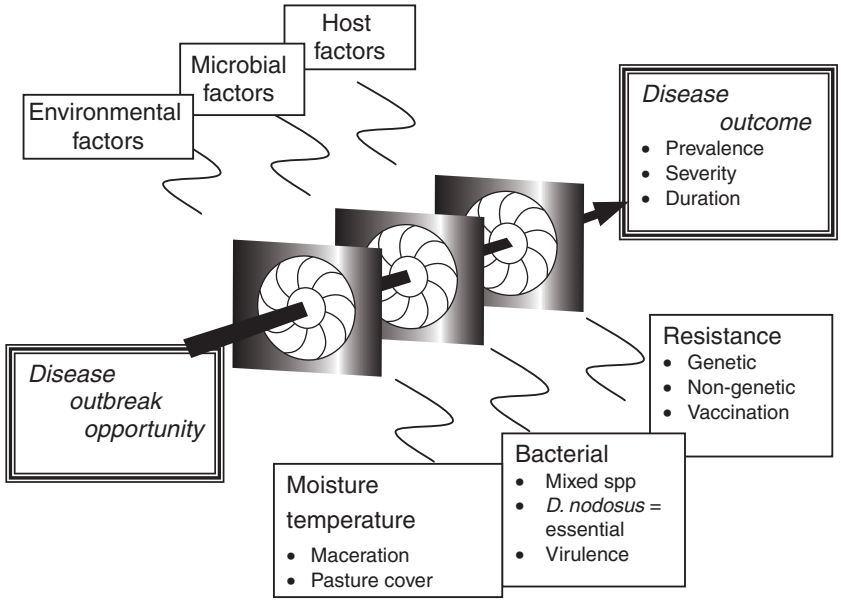

Fig. 12.2. Combination of host, microbial and environmental factors leading to the expression, severity and duration of footrot in a flock of sheep.

disease is feasible through direct manipulation of any of the three 'windows' of opportunity. This chapter will focus on those factors that affect host susceptibility only. Abbott and Lewis (2005) describe the non-host risk factors that determine the level of expression and severity of the disease.

Variation in resistance to infectious disease is the consequence of a combination of innate and acquired resistance. In the case of footrot, innate resistance may be responsible for preventing invasion of the epidermis by the bacteria responsible for the disease. O'Meara and Raadsma (1995) provide an overview of the physical and immunological factors of the host that aid or arrest the development of footrot.

The influence of non-genetic effects such as age, birth/rearing type, age of dam and sex of sheep on susceptibility to footrot has been reported. Adult sheep are more susceptible than lambs, and rams are more susceptible than ewes (Beveridge, 1941; Littlejohn, 1961; Raadsma *et al*., 1993, 1995; Woolaston, 1993; Nieuwhof *et al*., 2008b). Indeed Conington *et al*. (unpublished data) found that Texel ram lambs had footrot scores almost twice as severe as female lambs. Mclaren *et al*. (2008) reported differences in footrot scores in Blackface sheep between individual farms, litter size reared (with twin-rearing ewes having higher levels of footrot compared with single-rearing or barren ewes), grazing area and age of dam, with 6-year-old ewes having significantly worse footrot than younger sheep.

The combination of environmental, microbial and host factors on expression in footrot can be seen from variation between flocks. In a

recent footrot study in the UK reported by Conington *et al*. (unpublished data), there were wide differences in the percentage of sheep with foot scores that were greater than 0 (using the scoring system described above). For example, the average prevalence was 29% for 3600 Texel sheep that were scored on 19 farms, ranging from less than 1% to just under 60% (Fig. 12.3).

In general terms, acquired resistance is a sequel to either naturally acquired infection, colostral transfer of immunity or immunization. The identification of the principal causative bacterial agent, *D. nodosus*, and the immunogenic properties of *D. nodosus* fimbriae have made it possible to use vaccination as a method to control footrot. Following the initial development of whole-cell vaccines (Egerton and Burrell, 1970; Egerton and Roberts, 1971), recombinant deoxyribonucleic acid (DNA) techniques have now made it possible to produce effective vaccines comprised largely of *D. nodosus* fimbrial antigens (Egerton *et al*., 1987; O'Meara *et al*., 1993). To establish the importance of these antigens in vaccine formulations, the measurement of K-agglutinating antibody titres can be used as an indication of the ability of the vaccine to protect against infection with *D. nodosus* (Raadsma et al., 1994a).

Vaccines need to induce antibody titres against fimbrial antigens from each of the ten major serogroups of *D. nodosus*, since there is little or no cross-protection between serogroups, and field infections often involve infection with more than one serogroup. The problem of reduced titres, possibly due to antigenic competition in the host immune response (Raadsma *et al*., 1994b), limits the efficacy of multi-component footrot vaccines. The existence of genetic variation in response to active immunization against footrot is reviewed later in this chapter.

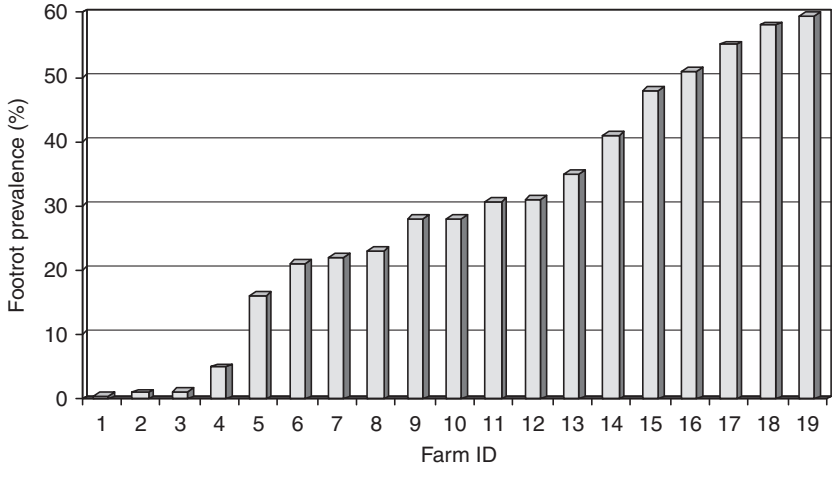

Fig. 12.3. Differences between farms in percentage prevalence of foot scores (sum of all four hooves) greater than zero. (Conington *et al*., unpublished data.)

Accepted control methods to minimize expression of footrot

Control of footrot has largely focused on elimination (eradication) of non-benign isolates of *D. nodosus* from flocks, prophylactic control through vaccination or control of affected sheep though topical and systemic therapy together with vaccination. None of these measures offers a long-term, easy-care approach to disease management. The full range of control methods of footrot in sheep, goats and deer have been extensively reviewed by Stewart (1989), Egerton *et al*. (1989), Abbott and Lewis (2005) and Egerton (2008b). Only aspects relevant to exploiting genetic variation in innate resistance or protective response to vaccination will be discussed further in this review.

Scope for Genetic Improvement of Resistance to Footrot

Incentive for genetic improvement in resistance to footrot

It is well recognized that in severe cases the cost of footrot to sheep industries can be very high. For instance, Nieuwhof and Bishop (2005) estimated the cost of footrot to the UK sheep industry at £24 million annually. However, in the absence of regulatory control, the occurrence of footrot on a farm does not always justify treatment. Egerton and Raadsma (1991) presented estimates of losses likely to arise when owners take one of three options: (i) take no action; (ii) implement control; or (iii) proceed through control to eradication. A number of important points were made:

- For mild footrot, the cost of control or eradication would exceed that which could be directly attributed to the disease if left untreated.
- The losses due to severe and moderate footrot could be halved through conventional control techniques, but the cost of control would be recurrent from season to season.
- The economic loss from severe or moderate footrot under a control management option is greater than that from uncontrolled mild footrot.

Breeding strategies could aim to reduce the impact of infection with virulent or intermediate isolates of footrot to that experienced with benign isolates of footrot, so that no specialized control strategies are warranted and the disease has minimal impact on production. An alternative strategy could be to improve the responsiveness of sheep to vaccination, so that footrot could be managed similarly to the clostridial diseases, with annual booster vaccinations offering effective protection.

Despite potential scope and incentive for breeding for increased resistance, alternative options for wide-scale preventative measures through flock eradication on a regional basis have proved to be extremely successful in some cases. For instance, the 20-year state-wide effort in New South Wales, Australia, with over 60 million sheep has seen the flock prevalence (proportion of flocks with footrot) decrease from over 15% to less than 0.1% of approximately 40,000 flocks (reviewed by Seaman, 2008). In the UK, on the other hand, the reluctance to embrace large-scale control programmes and apply preventative measures has

seen an endemic and systematic expression of footrot, with control primarily being short-term and focused on treatment of infected animals, especially those with severe lameness. Reasons for possible failure to adopt large-scale control methods in the UK have been reviewed by Abbott and Lewis (2005).

Resistance to footrot defined in general terms

Various diagnostic tests or indicators may be used to measure responses to challenge and hence infer footrot resistance.

Indicators based on clinical scores

Numerous measurement systems have been used to describe differences between sheep in their clinical response to footrot. Most of these systems are based on a simple binary scale indicating whether a foot or a sheep is affected or not. Sometimes the scale is extended beyond the two classes, in order to describe the severity of footrot as indicated by the extent of under-running of soft and hard horn of the foot. A number of footrot severity indices have been derived from individual foot scores. Raadsma *et al*. (1993) and Conington *et al*. (2008a) evaluated 22 primary and derived indicators of footrot severity, for their utility to describe differences between feet, between sheep and between flocks. For differences between sheep, all indicators were highly correlated (Raadsma *et al*., 1993; Conington *et al*., 2008a). Those traits with an ordered scale such as the number of feet affected are inherently more useful than scores or grades that do not reflect incremental levels of severity, as used, for example, by Skerman *et al*. (1988).

Indicators and liability to footrot

Resistance is often measured as an all-or-none trait. However, in reality, resistance is a multifactorial trait. The polygenic nature of the trait and all the non-genetic factors that influence expression of disease can be readily accommodated by adopting an underlying scale of liability, as proposed by Falconer (1965). This allows disease to be treated as a trait with quantitative characteristics similar to those of other production traits for which genetic parameters can be estimated. The genetic implication that all-or-none traits are due to single gene effects, where resistance behaves as a trait with Mendelian inheritance, rarely applies to bacterial diseases.

Of particular interest is how traits with multiple categories, such as number of feet affected or under-run, fit a threshold model. Similarly, the relationship between the threshold for becoming affected and the threshold for severe footrot (i.e. under-running) can also be examined under a multi-threshold model of liability. Raadsma *et al*. (1993) showed that for those indicators with several categories on an ordered scale (number of feet affected or number of feet under-run), increasing grades of severity reflect a single underlying variable.

Table 12.1 shows the proportion of expected foot scores under virulent, intermediate and benign outbreaks of footrot under conditions suitable for the expression of disease. As discussed above, resistant sheep are expected to

Table 12.1. Distribution of foot scores in different clinical forms of footrot expressed as percentage of sheep affected in virulent, intermediate and benign footrot. (Adapted from Egerton and Raadsma, 1991.)

	Clinical form of footrot		
Score	Virulent	Intermediate	Benign
4	10–70+	1–10	0–1
3	10–50	10–30	1–15
2	5–50	20–70	10–80+
1	5–10	5–10	5–10
0	30–50	30–60	20–80

show fewer feet affected, but also fewer feet with severe lesions, thus reducing the overall impact of disease from a virulent form to that approaching intermediate footrot and, in highly resistant flocks, similar to benign forms.

Serological indicators of resistance to footrot

It has been shown that antibodies specific to *D. nodosus* are generated during chronic and severe footrot infection (Egerton and Roberts, 1971; Egerton and Merritt, 1973; Fahey *et al*., 1983; Emery *et al*., 1984; Ferrier *et al*., 1986). Such antibodies may be directed to pilus or outer-membrane components. In addition, haemagglutination and protease-inhibiting antibodies have been detected following chronic infection (Egerton and Merritt, 1973). The results of these studies suggest that groups of sheep with more severe levels of infection develop higher antibody titres as a consequence of that infection. Raadsma *et al*. (1993) were the first to report positive correlations between *D. nodosus*-specific antibodies generated during infection and the susceptibility to footrot, on an individual sheep basis. Although antibody titre was correlated with severity and duration of infection, this relationship was not sufficiently strong (r = 0.3–0.6) to replace clinical scores as a phenotypic indicator to describe resistance in individual sheep.

Acquired immunity following vaccination with native or recombinant pili preparations is an important tool in the control of footrot. Immunity in this case is reflected by the absence of footrot following challenge, and by the level of antibodies directed against the protective pilus antigen, which is traditionally measured by K-agglutination (Egerton and Merritt, 1973). Raadsma *et al*. (1994c) showed a strong relationship between K-agglutinating antibody titre following vaccination and resistance following challenge.

The appropriate measures by which resistance in individuals can be measured are clinical scores of footrot lesions and K-agglutinating antibody levels following vaccination. The value of K-agglutinating antibodies in selection for innate resistance or responsiveness to vaccination is discussed below.

Repeatability of resistance

For both clinical assessment of footrot and measurement of K-agglutinating antibody titres, repeatability between and within operators is very high ($r > 0.9$;

Raadsma, unpublished data; Conington *et al*., 2008a), demonstrating that experienced operators can make consistent assessment of these two major indicators of resistance to footrot and response to vaccination respectively at any single time point.

As a consequence of the changes in footrot status of individuals following challenge and subsequent vaccination, clinical scores are moderately correlated when inspections are made at 2- to 3-week intervals (r = 0.31–0.70 prior to vaccination, and r = 0.02–0.31 after vaccination) (Raadsma *et al*., 1993, 1994a) and may be lower when intervals between inspections are longer, as reported for instance by Nieuwhof *et al*. (2008b) where the repeatability between scores obtained from twice-yearly inspections showed correlations ranging between −0.17 and +0.8 for various footrot scores. Therefore, the timing of inspections is important in the assessment of footrot and hence resistance. To get accurate assessments of footrot in individual sheep it is recommended to make a minimum of two inspections at least 3 weeks apart during an outbreak of footrot, to get a better assessment of footrot in individual sheep; this may need to be more frequent if year-round transmission of footrot is evident.

Using repeated measures of footrot status during an outbreak in the assessment of resistance has additional advantages in that repeatability models can be used in the estimation of genetic variation and prediction of breeding values. Raadsma *et al*. (1994a) showed that genetic correlations between footrot status at consecutive inspections in a flock of Merino sheep with very severe footrot were almost unity, thus describing the same genetic trait. Genetic correlations between footrot assessed before and after vaccination within the same outbreak were slightly lower (0.8; Raadsma *et al*., 1994a). Even lower genetic correlations (ranging from 0.18 to 0.55) were reported by Nieuwhof *et al*. (2008b) for inspections conducted in Scottish blackface and Mules in the UK on twice-yearly inspections and in generally lower prevalence of disease conditions. To adequately quantify resistance over time, repeated assessment of individual sheep may thus be necessary.

Also of relevance is the relationship between method of footrot challenge and resistance for challenges with different serogroups. Unfortunately, relevant data are scarce. Raadsma *et al*. (1994a) obtained phenotypic, genetic and environmental correlations between resistance following induced and natural challenge, which were (through necessity of experimental design) confounded with a serogroup of challenge strains. Phenotypic correlations between the responses to the two challenges were low (<0.10). Low negative environmental correlations (0.0 to −0.14) suggested that some carry-over effects may exist between sheep exposed to repeated challenge. Corresponding genetic correlations were moderate (0.37–0.67), suggesting that resistance under different challenge conditions may not be completely the same trait. Further data are necessary to determine whether this is due to the method of challenge or whether resistance is specific for different serogroups of *D. nodosus*. Similar results are likely to erode the accuracy of estimating genetic parameters from mixed field infections of footrot under semi-controlled expression, as reported by Nieuwhof *et al*. (2008b).

Documented genetic variation in resistance to footrot

Although for most major production traits the extent of genetic variation is reasonably well known, for footrot there is a paucity of information, except for Merinos (Egerton and Raadsma, 1991) and more recently detailed studies on Scottish blackface and Mules (Conington *et al*., 2008a; Nieuwhof *et al*., 2008b).

Differences between breeds

Limitations of the available literature describing breed differences in resistance to footrot were identified by Egerton and Raadsma (1991) and will not be covered further. In brief, most studies describing breed differences in response to footrot are confounded in that breed groups do not graze in mixed flocks and are thus unlikely to be exposed to the same challenge conditions. Further, in most cases the sample of sires used to generate the breed sample is sufficiently small that it is difficult to distinguish between sire or breed variation. Nevertheless, industry observations suggest that British breeds of sheep tend to have greater resistance to footrot than wool breeds such as Merino or Merino-derived breeds.

Differences between strains and bloodlines

As detailed by Egerton and Raadsma (1991), within the Australian Merino breed there are a number of distinct strains and bloodlines within strains. These strains and bloodlines within strains vary considerably in major production characteristics (such as fleece weight, fleece length, body weight), as well as in resistance to fleece rot and susceptibility to fly strike (Atkins and McGuirk, 1979; Raadsma and Rogan, 1987; Raadsma, 1991). In contrast, there is relatively little variation between strains or between bloodlines within strains for resistance to internal parasites (Eady *et al*., 1996).

Estimates for differences between major industry bloodlines of Merinos in their susceptibility to footrot are limited (Raadsma *et al*., 1994a). One relatively small investigation conducted by Raadsma, Swan and Purvis (unpublished data) involved 400 wethers from 11 fine- and medium-wool Merino bloodlines after separate exposure to an intermediate and then a virulent isolate of *D. nodosus*. Although no differences between the 11 flocks in resistance to the intermediate isolate were observed, substantial differences were observed following challenge with the virulent isolate. Repeated inspections over a 27-week period, following a challenge protocol similar to that used by Raadsma *et al*. (1994a), showed that the most resistant flock had 34% of sheep affected with severe footrot (score 3 or 4) compared with 79% for the most susceptible flock (Fig. 12.4). These results are interesting in that they highlight potential differences between bloodlines in susceptibility to severe footrot, which has not been recorded previously, presumably because in most flocks managers are actively trying to minimize the expression of footrot. Woolaston (1993) observed no difference in resistance to footrot in single-trait selection flocks selected for different levels of resistance to *Haemonchus contortus*.

Differences between sheep within flocks

Early estimates of heritability of resistance to footrot in various breeds have been summarized by Egerton and Raadsma (1991), who also detailed some of the

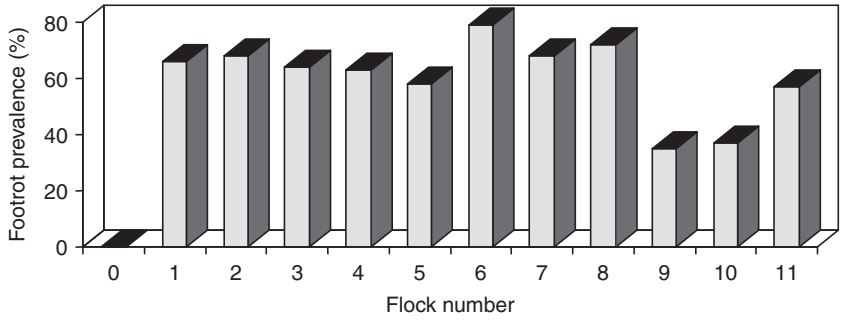

Fig. 12.4. Difference in footrot prevalence between 11 bloodlines (1–11) of Merino sheep born and raised contemporaneously and exposed to virulent footrot (n = 425).

problems associated with traits describing resistance to footrot. First, it is erroneous to diagnose 'footrot' only when under-running of soft horn occurs and 'footscald' when only IDS inflammation has been recorded, unless individual interdigital lesions were specifically confirmed to be free from *D. nodosus* infection. Failure to recognize this mars some studies. Second, most of the early estimates were from binomial data. In such cases, heritability estimates are dependent on the prevalence of the condition, which means that differences in heritability estimates could reflect differences in prevalence rather than differences in magnitude of genetic variation. However, it is possible to obtain estimates of the heritability of liability to footrot, independent of prevalence of the condition, through transformation of estimates on the observed scale (Falconer, 1989) or directly on the underlying scale (Gilmour *et al*., 1985). The difficulty in studying infectious diseases under uncontrolled conditions was highlighted by Woolaston (1993), who reported genetic differences between sire lines in prevalence and severity of footrot in Merino lambs following natural challenge. However, sire effects were confounded with paddock effects, which were of the same magnitude as the paddock effects in adult ewes allocated at random to sires.

Raadsma *et al*. (1994a) reported heritability estimates for eight clinical indicators of resistance to footrot (five describing the extent of clinical signs and three describing the extent of subsequent healing). Resistance was assessed in 1562 Merino sheep, representing the progeny from 162 sires in four major bloodlines, following exposure to virulent isolates of *D. nodosus* under both an experimental challenge in which footrot was induced and a separate natural (field) challenge involving a different isolate of *D. nodosus*. Resistance was assessed on seven occasions following induced challenge, and on five occasions following natural challenge. All sheep were vaccinated with primary and booster injections of a homologous rDNA pilus after initiation of the induced and natural challenge. Half-sib heritability estimates of resistance to footrot were low to moderate for single observations recorded pre-vaccination (0.07–0.22), and slightly lower for inspections made after vaccination (0.07–0.15). Genetic correlations among footrot indicators recorded at repeat inspections were high for observations pre-vaccination (0.87–1.00) and slightly lower for observations made after vaccination (0.52–1.00). Heritability estimates derived from repeat measurements

approached 0.30 for most indicators, except for indicators describing healing, which had a heritability of almost zero. Heritability estimates of liability to footrot ranged between 0.09 and 0.41, depending on the time after challenge when the inspections were made. The genetic correlation between induced and natural footrot ranged from 0.14 to 0.95, depending on the period over which inspections were made, with an average of 0.67. It was concluded that there is substantial genetic variation within flocks of Merino sheep in resistance to challenge with virulent isolates of *D. nodosus*, especially if resistance is assessed regularly, for example three inspections at 3-week intervals (Table 12.1).

Summarizing observations made by Raadsma *et al*. (1994a) and Nieuwhof *et al*. (2008b) it can be concluded that:

- Heritability of resistance based on a single inspection is generally lower than estimates based on multiple inspections during the same challenge.
- Heritability of resistance measured after vaccination is usually lower than heritability of resistance based on inspections before vaccination.
- Heritability of resistance to induced footrot and natural footrot are similar, but the genetic correlations between the two are not (although the type of challenge was confounded with serotype of infection).
- Heritability in very young lambs of hill breeds is very low and optimal time of selection for resistance requires additional investigation.
- Heritability of resistance based on expressed prevalence of footrot will change according to prevalence of the disease.
- Resistance based on an underlying scale of liability (risk) to footrot shows a moderate heritability (0.25–0.30), indicating that this trait should respond to selection.
- Normal quantitative breeding practices could be adopted to exploit genetic variation in resistance to this disease based on conventional selection and breeding practices, provided animals are exposed to challenge.

It can be concluded that there is substantial genetic variation within flocks of sheep in resistance to challenge with virulent isolates of *D. nodosus*. However, this requires individuals to be exposed to direct challenge. The genetic variability among a group of sires can be expressed as estimated breeding values (EBVs) based on performance of progeny under direct challenge. Based on these observations it was shown that sires showed genetic differences in resistance in contrasting breeds and environments (Fig. 12.5) and it would possible to select breeding replacements with high resistance to footrot without direct exposure of valuable breeding individuals to footrot. The use of progeny data can also be incorporated in sire evaluation across commercial evaluations as part of industry-wide evaluations in a broad range of traits, as is currently practised worldwide. Alternatively for the UK, it has been recently recommended that regular (annual) foot screening of breeding ewes be used to produce EBVs for progeny (partly since heritability in adult sheep may be higher than in lambs and also because the majority of offspring are sent for slaughter before 4 months of age before the main period of high footrot prevalence, which is usually in the wetter months of autumn and winter). Breeders of 'maternal' ram lambs already have EBVs for fertility and other traits measured on ewes only

Table 12.2. Summary of heritability estimates from footrot inspections in Merino, Mules and Scottish blackface (SBF) sheep following natural and experimentally induced footrot.

	Study 1[a]	Study1[a]	Study 2[b]	Study 2[b]
Breed	Merino	Merino	Mules	SBF
Challenge conditions	Induced	Natural	Natural	Natural
Mean of multiple inspections during challenge – before vaccination	0.20 (s.e. 0.06)	0.18 (s.e. 0.06)	0.12 (0.11)	NA
Mean of multiple inspections during challenge – after vaccination	0.07 (s.e. 0.06)	0.09 (s.e. 0.04)	NA	NA
Repeatability model using multiple observations – before vaccination	0.27 (s.e. 0.07)	0.29 (s.e. 0.07)	0.13 (s.e. 0.07)	0.05 (s.e. 0.02)
Repeatability model using multiple observations – after vaccination	0.16 (s.e. 0.08)	0.28 (s.e. 0.07)	NA	Na
Underlying scale of liability to footrot estimated using threshold model	0.28	0.30	0.26 (s.e. 0.11)	0.19 (s.e. 10)

[a]Study 1 – described by Raadsma *et al*. (1994a); all animals with foot scores >1 were included as affected.
[b]Study 2 – described by Nieuwhof *et al*. (2008b); all animals with foot scores >1 were included as affected.

that have been estimated from their adult female relatives. So, including footrot in this category is a logical extension of this breeding design, and also means that valuable breeding rams need not themselves be exposed to footrot.

Skerman (1985) and Skerman and Moorhouse (1987) reported the development of a bloodline with increased resistance to footrot, in both the Romney Marsh and Corriedale breeds. Both bloodlines evolved through direct selection under natural outbreaks of footrot and extensive use of sires whose progeny showed increased resistance over their contemporaries. Both studs claimed that, as a consequence, footrot was an insignificant problem in their flocks (Skerman and Moorhouse, 1987; Warren *et al*., 1990). Although reports of this nature highlight the potential for genetic control of this disease, formal genetic comparisons such as those described by Skerman and Moorhouse (1987) are needed, but are usually lacking, in on-property experiments. The follow-on benefit of increased usage of breeding stock from more resistant bloodlines in the Merino industry still awaits evaluation, but has already prompted certain stud breeders to place heavy selection pressure on resistance (Patterson and Patterson, 1989, 1991).

In the UK several farmers have adopted the approach of identifying and culling footrot-affected sheep, to reduce footrot prevalence in their flocks. Even though this can be an effective strategy coupled with management practices to minimize footrot prevalence in their own flock, there is no guarantee that this greater resistance will persist when breeding stock are moved to other farm environments. Indeed, they may even be immunologically more susceptible to footrot if their own environment is largely footrot-free. Such a practice is commonplace in the UK, where the sheep industry is characterized by a high dependency on bought-in breeding stock and ram sharing. The use of EBVs for resistance to footrot that are generated as part of a structured breeding programme (e.g. Sire Reference Scheme, where participating flocks are genetically 'benchmarked' with others through genetic links created from the use of

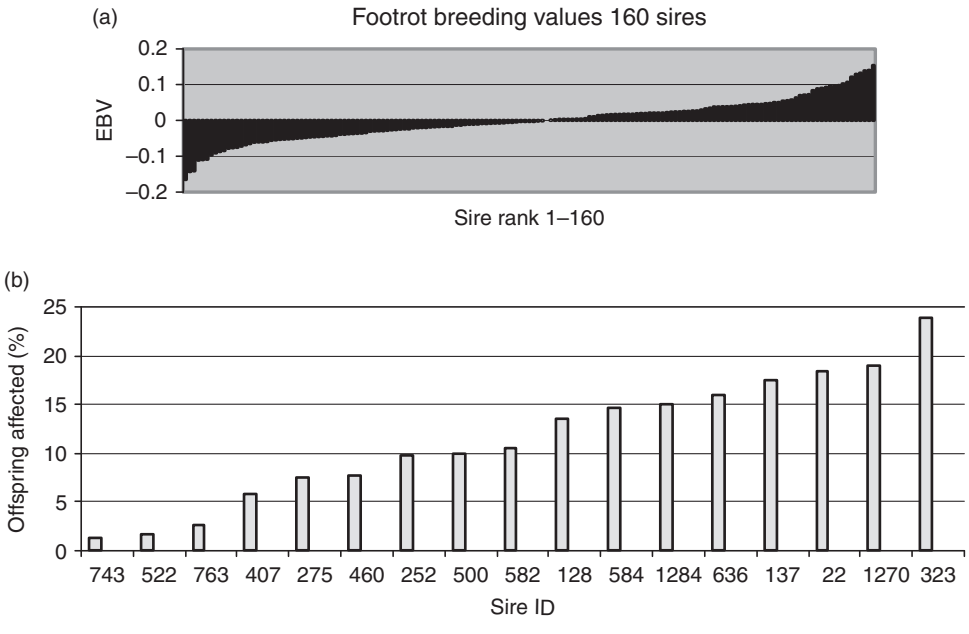

Fig. 12.5. (a) Distribution of estimated breeding values (EBVs) for footrot resistance in 160 Merino sires (in Australia) based on progeny performance testing under direct challenge with virulent footrot. Negative EBVs indicate higher resistance; positive EBVs indicate lower resistance. (b) Differences between blackface sires in the number of progeny affected by footrot from a single flock in Scotland. (From Conington *et al.*, 2008a.)

common sires) will effectively overcome the limits of within-farm selection for footrot, give greater confidence and reduce risk associated with the selection of resistant rams. The use of EBVs for footrot resistance for the Texel breed is in discussion, and it is anticipated that the sale of rams with footrot EBVs would greatly enhance their marketability.

Genetic variation in immunological response to *D. nodosus* antigens

Immunological responsiveness to *D. nodosus* antigens is relevant in three distinct scenarios. First, immunological host responses generated during the course of an infection are a valuable objective indicator of resistance during or immediately after infection. Second, response following vaccination in non-challenged animals is potentially a valuable means of indirect selection for resistance without the need for direct challenge. Third, vaccination with *D. nodosus* immunogens represents an effective method of footrot control (as described above), and response to vaccination may be amenable to genetic improvement to enhance vaccine efficacy. Raadsma *et al*. (1994a,b, 1995, 1996) have addressed each of the three potential applications of immunological responsiveness in breeding for resistance to footrot. A summary of their findings is presented below.

Immune response as an alternative clinical indicator during infection

Despite the consistent positive phenotypic correlations between antibody titre and footrot score, estimates of the genetic correlations and heritability suggested that measures of antibody level during infection were not suitable alternative indicators to footrot scores. Efficiency of selection based on serological indicators during infection would be only 50% of that based on clinical scores.

Vaccine response as an indirect selection criterion

Vaccine responses to *D. nodosus* antigens measured in sheep free from challenge show low genetic correlations with resistance to footrot in non-vaccinated sheep under either homologous (challenge serogroup same as vaccine antigen) or heterologous serogroups. Despite the higher heritability for vaccine response than innate resistance, indirect selection for innate resistance based on vaccine response in non-challenged sheep has little potential because of the low and inconsistent genetic correlations between the two traits. Efficiency of selection based on response to vaccination in the absence of challenge is expected to be less than 20% of that based on direct selection. O'Meara and Raadsma (1995) reviewed other immunological indicators of resistance to footrot with a view to assessing their potential as alternative indicators, but found none to be more consistent than K-agglutinating antibody levels.

Protective immune response following vaccination

Large phenotypic and genetic differences were recorded for antibody responses to nine out of ten major serogroups of *D. nododus* commonly used in multivalent footrot vaccines. Based on a sample of 120 half-sib sire families comprising a total of 1200 progeny, considerable variability in heritability estimates was observed for antibody responses to each of the nine serogroups. The estimates ranged from 0.24 to 0.58 (s.e. 0.08–0.12) and were independent of mean antibody level. The range in estimated breeding values for a sample of sire groups showed that the difference between the lowest and highest ranked sire represents a greater than twofold difference in mean antibody titre following vaccination. Results suggest that genotype of the host confers a differential capacity to respond to individual vaccine antigens when multiple antigens are presented simultaneously in the same vaccine.

Genetic heterogeneity in vaccine response

Despite 34–78% homology of the amino acid sequence among the fimbrial subunits of the nine *D. nodosus* fimbrial proteins, each elicits a serogroup-specific antibody response that offers almost no cross-protection between serogroups. Examination of genetic correlations among antibody response to each serogroup revealed considerable heterogeneity, ranging from moderately negative (–0.40) to strongly positive (+0.87). Utilizing procedures described by Raadsma *et al*. (1996), genetic control of antibody response to *D. nodosus* antigens was partitioned into that which was shared (common) and that which was serogroup-specific. On average, 32% of antibody response was under

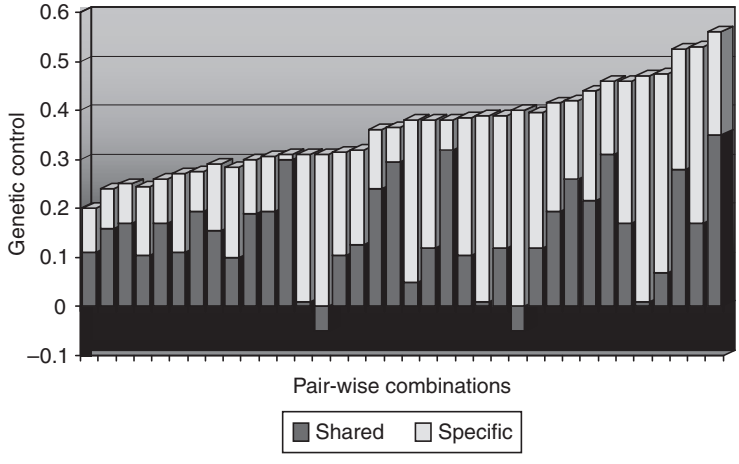

Fig. 12.6. Degree of genetic control in antibody responsiveness following a nanovalent *Dichelobacter nodosus* serotype vaccine. Degree of shared and serogroup-specific control determined from genetic variance and covariance of all combinations of antibody responsiveness. (Adapted from Raadsma *et al.*, 1996.)

shared genetic control, but the actual genetic correlations ranged from 0% to 87%, depending on the combination of antigens. This is shown in Fig. 12.6. It was concluded that significant additive genetic variation exists in Merino sheep for response to *D. nodosus* vaccines. However, there was no strong evidence for common genetic control of immune response to different antigens, and each serogroup may require a unique combination of immune-response genes in the host for optimum vaccine performance. In Fig. 12.6 the common genetic control of response to different antigens is shown. The genetic control of vaccine response is important not only in response to multivalent vaccines, but also more recently for the capacity to target vaccines for specific serogroups on the farm (Dhungyel *et al.*, 2008). Although vaccination is used as a means to facilitate eradication of each serogroup, it is important that appropriate host responses to all potential antigens are not compromised.

Genetic markers for resistance and vaccine responsiveness

The option of exploiting genetic differences in footrot resistance through direct selection is often of limited practical value, as it requires large numbers of animals to face challenge. The possibility of using indirect selection strategies is therefore appealing. The limited utility of serological responses as indirect selection traits was discussed above. No other physiological responses have been identified that show potential as indirect selection criteria. Other options for indirect selection strategies include the use of genetic markers linked to resistance genes. The role of the major histocompatibility complex (MHC) in

modulating immune responses and subsequently disease resistance is well documented for a number of species. MHC gene products are glycoproteins present on the surface of some cells, and are divisible into two types, Class I and Class II histoglobulins. Genetic polymorphism within both the Class I and Class II regions has been investigated in relation to innate resistance to footrot, and response after vaccination with *D. nodosus* antigens (Outteridge *et al*., 1989; Litchfield *et al*., 1993; Escayg *et al*., 1997). Raadsma *et al*. (1998) reported preliminary findings from genome screens in resource flocks held at AgResearch (NZ), in relation to response to vaccination with *D. nodosus* antigens. The results suggested that a region in the MHC plays a role in regulating serogroup-specific responses, and a region on chromosome 1 contributes to variation in generalized vaccine response.

Work at Lincoln University, New Zealand, has described allelic variation within the ovine MHC class II region, specifically at the *DQA2* gene (Hickford *et al*., 2004), and this information was used to develop genetic markers for footrot resistance. This led to the implementation of a genetic test for footrot resistance that is currently being used in New Zealand ram-breeding flocks, and it is estimated that over a million progeny have been born to footrot-genotyped and accordingly selected rams (Abbott *et al*., 2007). Research has been conducted in the UK to establish whether the existing knowledge of alleles associated with footrot susceptibility in New Zealand can also be used for UK sheep breeds. Footrot records and DNA from blood samples collected from blackface and Texel sheep were screened for known polymorphisms of the *DQA2* gene, according to the methodology already developed in New Zealand. The information generated has been used to associate the severity of footrot with the genetic marker. As it is plausible that other MHC genes may also play a role in the ability of sheep to resist footrot infection, this research also investigated the predictive power of a multiplexed set of 12 microsatellite markers that spanned chromosome 20 (courtesy of Jill Maddox, University of Melbourne). These markers, combined with the DQA2 results, potentially provide information on the footrot susceptibility of Texel and blackface sheep.

Overall, the conclusion is that associations between resistance/antibody response and MHC polymorphism are not sufficiently strong or consistent to justify their application in the field unless fully validated. Obviously, the search for suitable markers should be extended to other regions within or outside the MHC. The use of genome-wide screens with high-density coverage of single nucleotide polymorphisms (SNPs) is now feasible in sheep (http://www.sheephapmap.org). This opens up the use of genome-wide selection (GWS), as has been reviewed in dairy cattle (Goddard and Hayes, 2007; Raadsma *et al*., 2007b) and has been applied for disease resistance such as mastitis resistance in dairy cattle (Raadsma *et al*., 2007a). The potential accuracy of such genome-wide molecular indices or molecular breeding values (MBVs) finally may make indirect selection without challenge data reality. To develop such MBVs requires access to DNA repositories of well-characterized animals, and this currently limits the application in resistance to footrot.

Resistance and other breeding objectives

Should it prove to be feasible to improve resistance to footrot through selective breeding, it is unlikely to be the sole breeding objective. It is important, therefore, to have accurate information on the correlations between resistance to footrot and all the major production traits recommended as breeding objectives and/or selection criteria for sheep and wool production. In high-rainfall areas, resistance to other important diseases such as fly strike and internal parasites may also need to be considered.

Relatively few estimates of phenotypic or genetic correlations between resistance to footrot and production traits are available. Nieuwhof *et al*. (2008a) demonstrate the complexity of establishing relationships between production and resistance to footrot in the face of a disease challenge and supported the earlier observations of Raadsma *et al*. (unpublished data) in the case of footrot resistance and body weight. A few important observations were made, namely that sheep with severe footrot suffered weight loss in the range of 0.5–2.5 kg during a 6- to 9-week outbreak; almost all sheep showed signs of compensatory growth in body weight, so that at the end of an outbreak followed by treatment sheep had regained their projected body weight; heavier sheep tended to have a greater predisposition to footrot; sheep with a greater genetic capacity for growth suffered relatively lower body weight losses than sheep with a lower genetic merit for growth; and rate of body weight loss in the face of challenge had a genetic component (15–30% heritable). Estimates of genetic correlations between resistance and production need to be obtained for production traits under both challenged and non-challenged conditions, to account for the environmental influence of disease on performance. The results indicate no strong undesirable genetic correlations. In fact, all estimates of correlations with important production traits (clean fleece weight and mean fibre diameter) were neutral (range −0.1 to +0.2).

Mclaren *et al*. (2008) reported genetic and phenotypic parameters for footrot with other breeding goal traits that are currently used for some hill breeds in the UK. These included lamb growth and carcass traits, as well as maternal traits of pre-mating live weight, longevity, lamb survival, number of lambs reared, maternal ability and fleece weight. All phenotypic correlations were less than 0.04 and, even though the precision of the genetic parameter estimates was generally low, they concluded that the inclusion of footrot into multi-trait trait breeding programmes is unlikely to affect the performance in other traits significantly.

Taken together, these data suggest that selection for important production traits will have no adverse or desirable effect on resistance to footrot. Similarly, selection for increased resistance to footrot will not adversely affect important production traits, and there should be sufficient scope to improve production and resistance simultaneously, albeit at slower rates, than if either objective was taken as a sole breeding objective. The environmental effects on fleece quality characteristics such as staple strength or reproductive success, however, are not recoverable, and represent a potentially large production penalty of having footrot in flocks of sheep.

Resistance to other diseases

The question of broad-based resistance (i.e. resistance to multiple diseases) is relevant here, since the important diseases in sheep production are often influenced by common environmental factors. Raadsma *et al*. (1997) reported a study in which resistance to several important diseases that affect production was examined in the same flock under the same environmental conditions. Genetic variation for resistance to each of the diseases existed within the flock, but genes conferring resistance to one disease did not, in general, affect resistance to other diseases, as implied by finding that genetic correlations between resistance to different diseases were low. The only possible exception was a moderate (undesirable) genetic correlation between resistance to fleece rot (a major predisposing factor to blowfly strike) and resistance to footrot.

The observation on a neutral genetic correlation between resistance to footrot and resistance to internal parasites confirms earlier observations by Woolaston (1993), who reported a low genetic correlation (0.02 ± 0.20) between resistance to footrot and resistance to a major internal parasite in sheep, namely *H. contortus*. Similarly, Gray and Woolaston (1991) did not observe significant differences in K-agglutinating antibody levels following vaccination with a whole-cell *D. nodosus* vaccine in flocks with various levels of resistance to *H. contortus*.

It is likely that breeding programmes aimed at resistance to multiple diseases will need to consider each relevant disease separately. It may be feasible to exploit and combine resistance to multiple diseases, including footrot, from different flocks that have been selected specifically for resistance to just one disease. This type of breeding exercise would be greatly assisted by genome-wide selection.

Conclusion

A primary requirement for inclusion of resistance to footrot in breeding programmes is knowledge of the extent of genetic variation in resistance and the genetic correlations between resistance and all other important traits, including production and resistance to other diseases. At the moment, there is sufficient preliminary information available on all relevant genetic parameters to include resistance to footrot in sheep-breeding programmes. The main challenge for animal breeders is to decide on the appropriate weighting for resistance to footrot and scope for control through alternative and complementary strategies. In many cases the cost of footrot will vary pending environmental and microbial factors, and the cost of control and economic impact will vary considerably. The use of sire reference information where progeny groups are maintained under natural field challenge can provide additional information on resistance without specifically challenging valuable breeding animals. The use of indirect selection traits either through physiological indicators or DNA markers is not sufficiently robust to be universally applicable, although the advent of genome-wide selection offers prospects for accurate genetic indicators to be established in the appropriate breeds and environments.

Finally, it is not sensible to consider selective breeding programmes to improve resistance to footrot, or other important diseases for that matter, without also considering non-genetic forms of disease control. An overall animal health programme will need to consider all avenues that are economically feasible and may need to improve management, as well as incorporating disease resistance in selective breeding programmes.

With the development of vaccines, there is a need for animals to respond to a large number of clearly defined immunogens. Genetic restrictions that prevent an adequate response to multivalent vaccines need to be identified and investigated for possible modification through selective breeding. The use of footrot as a model host–vaccine response system may show how this could be done for other species and other veterinary vaccines.

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IV Parasites and Vectors

13 Breeding for Resistance to Nematode Infections

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Summary

Efficient and welfare-friendly livestock production requires the control of nematode infection. Treatment with anthelmintics is the most widely used option, but this is threatened by the evolution of drug resistance in parasite populations. The exploitation of genetic resistance is an attractive method for nematode control. There are three strategies for utilizing genetic resistance: use of resistant breeds, cross-breeding and selective breeding. Resistant breeds of cattle and sheep have been identified but more research is needed, especially on cross-breeding. Selective breeding is most advanced in Australian and New Zealand sheep and remarkable results have been achieved. There is no doubt that selective breeding is an attractive option for nematode control.

Genetic resistance should be utilized without further delay. Future developments may help to simplify and accelerate the adoption of genetic methods. Three areas that are likely to see further developments are: determining the distribution and nature of susceptibility to nematode infection and disease; better identification of resistant animals; and understanding constraints on the development of protective immunity.

Introduction

Although the parasitic nematodes of each livestock species are often considered as a single entity, they form a number of distinct parasite communities. Among sheep in cool temperate areas such as Scotland there can be up to a dozen or so different nematodes species in the gastrointestinal tract, but the community is dominated by the small, brown stomach worm *Teladorsagia circumcincta* (Stear *et al*., 1998). In hot tropical and subtropical areas such as the southern USA and much of Australia, the nematode community is predominately *Haemonchus contortus*. In the warm temperate climate of New Zealand, two nematode species dominate: *Trichostrongylus colubriformis* and *Teladorsagia circumcincta*. In cattle, *Ostertagia ostertagi* dominates in temperate climates such as the UK and north-eastern USA, whereas the genus *Haemonchus*

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(especially *H. placei*) is the major problem in hotter areas such as Queensland and the Northern Territory of Australia. Although these communities have much in common there are important differences among them (Stear *et al*., 2007a).

One shared feature of parasitic nematode communities is their deleterious effect on livestock. Uncontrolled nematode infections can cause disease and death, but perhaps their main economic effect is to reduce livestock production. In the UK, nematodes are responsible for almost £100,000,000 in lost sheep production each year (Nieuwhof and Bishop, 2005) and are the most costly endemic disease of sheep. In Australia, nematodes were estimated to reduce live weight gain in young Merino sheep by 38% and wool production by 7% (Gray and Gill, 1993; Albers *et al*., 2000). However, the pathogenesis of nematode infection is complex and differs among species (Stear *et al*., 2003). The intensity of infection is clearly important, but other factors such as relative nutritional and immunological status as well as concomitant infections also influence the severity of clinical and subclinical infection.

None the less, efficient and humane livestock production demands that nematode infection be controlled. There are several methods that have been advocated for gastrointestinal nematode control (Sayers and Sweeney, 2005; Stear *et al*., 2007a). The mainstay of current control methods is anthelmintic treatment which is cheap, simple and effective. However, the sustainability of this method is threatened by the evolution of drug resistance in parasite populations (Bartley *et al*., 2003). An alternative method is grazing management, which can help to control infections but is often impractical and insufficient on its own. Nutritional supplementation can be effective but is expensive. Additionally, supplementation is awkward for sheep or cattle reared at low density under extensive management schemes. Administration of fungi that feed on nematodes can be as effective as drug treatment, but currently this option is limited by the need to administer fungal spores every day. Vaccination holds great promise but there are no vaccines currently available. Perhaps the most promising alternative or supplementary control method is the use of genetic resistance.

Use of Genetic Resistance

There are three methods of utilizing genetic resistance for disease control: selection among populations, cross-breeding and selection within populations (Nicholas, 1987). These methods are not mutually exclusive. For example, breeders can select within the populations that farmers use for cross-breeding.

Selection among populations essentially means the identification of genetically resistant breeds and the substitution of animals from susceptible breeds by their more resistant contemporaries. There are large differences among breeds of cattle and sheep in resistance to nematode infection, although most of this research has been done with *Haemonchus* species. For example, the Red Maasai sheep of East Africa are more resistant than imported breeds (Baker *et al*., 1993, 1994, 1999; Baker, 1994; Mugambi *et al*., 1996), while several resistant breeds have been identified in south-eastern USA and the

Caribbean islands, including the Gulf Coast native (Bahirathan *et al*., 2000; Burke and Miller, 2002), the St Croix (Gamble and Zajac, 1992) and the Barbados blackbelly (Yazwinski *et al*., 1979, 1981). In Scotland, Scottish blackface sheep are more resistant to nematode infection than Finn-Dorset or Hampshire sheep (Abbott *et al*., 1985; Wallace *et al*., 1995, 1996, 1998, 1999). There are also differences among cattle breeds. In northern Australia, cross-bred cattle have been introduced in an attempt to minimize the ravages of tick infestation. These cross-bred animals are predominantly crosses between *Bos indicus* breeds originally from Asia and *Bos taurus* breeds from Europe. In addition to greater resistance to the cattle tick *Boophilus microplus*, these animals are often more resistant to nematodes.

While breed substitution is simple and effective, it has seldom been used to control nematodes, largely because of concerns over productivity. For example, Merino sheep are relatively susceptible to nematodes but their wool production dwarfs that of other breeds and, given a stable wool market, there is no economic advantage to Australian farmers in switching breeds. In the southern USA, sheep are often reared by children for 4H agricultural shows. Here it is sensible to use resistant sheep which thrive in conditions that kill lambs from susceptible breeds. However, this is a small market. Even in East Africa where careful comparisons have shown that Red Maasai sheep are more productive than sheep of the more susceptible Dorper breed under humid or sub-humid conditions (Baker *et al*., 2003), the larger Dorper sheep are often preferred because tradition and courtesy dictate that large sheep are killed for religious feasts.

Cross-breeding has not been explored as a method of nematode control. However, many production systems, especially in cattle and sheep production in the UK, use cross-bred animals. These systems have been widely adopted because they offer superior production. It is possible and perhaps even likely that the superior production is in part because of better disease and nematode control.

The main use of genetic resistance is in the selective breeding of resistant sheep. One approach to selective breeding is to demonstrate the feasibility, estimate the desirability and explore the sustainability (Stear *et al*., 2001a). The feasibility is shown by the existence of heritable variation, i.e. that the heritability is significantly greater than zero. Studies in sheep from Australia (Windon and Dineen, 1981; Albers *et al*., 1987; Woolaston and Windon, 2001; Pollott *et al*., 2004), New Zealand (Bisset *et al*., 1992; Douch *et al*., 1994; Morris *et al*., 1997, 2004, 2005; Morris, 1998), France (Bouix *et al*., 1998; Gruner *et al*., 2002, 2004) and the UK (Bishop *et al*., 1996; Stear *et al*., 1997a) have clearly demonstrated the heritability of faecal egg counts as a marker of resistance to nematode infection. These results apply to all communities of nematodes. Even more convincing demonstrations have come from selection experiments in Australia and New Zealand. Deliberate infection with *H. contortus* (Woolaston *et al*., 1990) or *T. colubriformis* (Woolaston and Windon, 2001) in Australia and natural mixed infections in New Zealand (Morris *et al*., 1997; Bisset *et al*., 2001) have produced sheep with enhanced resistance to gastrointestinal nematode infection. The selective breeding of

nematode-resistant sheep is now established in the sheep industry (Karlsson and Greeff, 2006). There is no doubt that selective breeding of nematode-resistant sheep is feasible.

The situation is similar in cattle. Here, research on genetic resistance has been reported from Australia (Stear *et al*., 1988, 1990; Mackinnon *et al*., 1991), Africa (Ross *et al*., 1960), South America (Suarez *et al*., 1990) and the USA (Leighton *et al*., 1989). The heritabilities are similar to those reported in sheep and, similarly, there is no doubt that selective breeding of nematode-resistant cattle is feasible.

The concentration of nematode eggs in the faeces has been used as the selection criterion and this is relatively straightforward. Farmers, veterinarians or consultants can collect faeces and either count them at the site of collection using special kits or send them to a laboratory for counting. There are a couple of points to note. First, genetic resistance is only apparent following prolonged infection for several weeks. Second, the heritability of a single egg count is variable but generally in the range of 0.2–0.4; counting multiple aliquots from the same sample or multiple samples taken at different times can substantially increase the heritability and hence the response to selection (Stear *et al*., 1990).

The desirability of breeding for disease resistance depends upon the relationships with productivity. The most suitable measurement in a genetic context is the genetic correlation, which is the correlation between the true breeding values for the different traits. An unselected trait will respond to selection on a correlated trait. There are differences among the estimated genetic correlations from different countries (Morris *et al*., 2005). In New Zealand, there are unfavourable genetic correlations between faecal egg counts and live weight gain, fleece weight and breech soiling (Morris *et al*., 2000a). In Australia, the genetic correlations are much weaker. In contrast, the genetic correlations between live weight and faecal egg count are strong and favourable with natural, mixed, predominantly *T. circumcincta* infections in Scotland (Bishop *et al*., 1996) and Poland (Bouix *et al*., 1998). In Scotland, the size of the genetic correlations changes as animals mature and immune responses develop (Davies *et al*., 2005). In Australian cattle, the genetic correlations between growth and faecal egg count vary between the wet and dry seasons (Mackinnon *et al*., 1991).

The reason for the variation in the estimated genetic correlations is unclear, although there are several possible explanations. Obviously, differences in the breed of the host, the composition of the parasite community and the way animals are managed could explain the apparent inconsistencies among the results. The effect of nematodes on production is related to the intensity of parasite infection. The intensity of infection is likely to vary among studies and this could influence the genetic correlations. In particular, the differences between the wet and dry season could reflect the greater intensity of infection in the wet season. In addition, the European studies generally looked at animals in their first grazing season while the studies from Australia and New Zealand generally looked at older animals. There appear to be two major mechanisms of immunity to nematodes: an IgA-mediated response that reduces worm growth and fecundity and an IgE-mediated immediate hypersensitivity response that controls the number of nematodes (Stear *et al*., 1997b, 1999a,b).

The immediate hypersensitivity response develops later and is likely to be more pathogenic (Stear *et al*., 2003). Therefore, resistant animals could show favourable genetic correlations with productivity in the first grazing season but these could turn unfavourable as animals mature.

However, there is an added epidemiological bonus when selecting livestock for disease resistance. Resistant animals produce fewer eggs and the contamination of the environment is reduced. Mathematical models have shown that the reduction in egg counts due to this epidemiological response substantially improves the effective response to selection (Bishop and Stear, 1997). This means that a flock or a herd of relatively resistant animals is exposed to lower rates of infection. Consequently, the productivity of animals in resistant herds is often higher than in animals from susceptible flocks. Therefore, there is little doubt that selective breeding for nematode resistance is desirable.

The sustainability of breeding for disease resistance is more controversial. Sustainability involves predicting the future, but the future is unknown and in the absence of facts controversy flourishes. Essentially there are two lines of evidence and one argument about sustainability. First, the existence and persistence of disease-resistant breeds demonstrates that disease resistance is inherently stable and potentially sustainable. Second, evidence from selective breeding of livestock in commercial and research situations indicates that parasites do not readily subvert genetic resistance. These results suggest that genetic resistance is stable, for at least several decades. The argument is more theoretical. In the natural world, the host–parasite relationship is constantly changing as parasites and hosts evolve. The selective breeding of livestock merely restores some balance to the relationship. To this extent, breeding livestock for disease resistance is merely attempting to mimic evolution. There is a case for breeding carefully and continually monitoring the results but there is no coherent case for doing nothing. Therefore, there is no reason to doubt the sustainability of breeding for disease resistance.

Future Developments

The prospects for sustainable control of parasitic infection are likely to be enhanced with a more detailed understanding of the host–parasite relationship. To develop this detailed understanding is a long, hard undertaking that requires effective collaboration among scientists from a variety of disciplines, including parasitology, genetics, immunology, epidemiology and ecology. Scientists trained in different disciplines often interpret the same phenomena differently. Reconciling these different perspectives is an active area of research. In the future, we hope to see the different perspectives coalesce.

The distribution of susceptibility to disease

One area of active research is the distribution among hosts of susceptibility to disease.

For most medical, veterinary and wildlife parasites, the number of parasites present in each individual host is highly variable. Similarly, the number of potentially infective transmission stages shed from each host also differs among individuals. However, scientists from different disciplines interpret this variation quite differently – depending upon their training and on the focus of their enquiries.

Genetic theory suggests that the distribution of parasites among individuals reflects the contribution of polymorphic genes, variable environmental factors and the interactions among them. For geneticists, anything that is not genetic is classified as environmental. Environmental factors include sex, age, time of sampling and previous history of exposure. In most diseases, the variation among individuals in susceptibility (which is often called liability by quantitative geneticists (Falconer and Mackay, 1996)) cannot be measured directly. Therefore, quantitative geneticists have assumed that liability follows a normal distribution, and that disease develops when some threshold of liability is exceeded (Falconer and Mackay, 1996). Under these assumptions, those individuals that develop disease are those that are genetically predisposed or those that have an unfavourable combination of environmental circumstances (high levels of exposure, poor nutrition and so on) or some combination of unfavourable genetics and unfavourable environment. The number of parasites among hosts is not usually normally distributed. Consequently, some geneticists transform the data before analysis (Bishop *et al*., 1996). However, this is merely a statistical convenience.

Parasitologists prefer to talk about susceptibility rather than liability, and they define susceptibility differently from geneticists. Many parasitologists assume that susceptibility to disease is related to the number and size of parasites in an individual. Consequently, the distribution of susceptibility is overdispersed because it follows the distribution of parasites. Other factors, such as nutrition and co-infection, also influence the development of disease (Sykes and Coop, 2001).

In contrast, a purely ecological perspective places greater emphasis on the interactions among different species and between species and the environment. The distribution of parasites among hosts is seen not only as a function of parasite infectivity and host susceptibility, but also to depend on the demographic features of both the host and parasites. In this view, the age of the host at the time of infection, the history of exposure to infection and the variety and density of parasite species within the host might all influence the observed distribution of parasites among hosts. Host susceptibility is therefore seen as a dynamic phenomenon in contrast to the more static view often taken by geneticists and parasitologists.

The distribution of parasites and of susceptibility has implications for the design of experiments as it affects the precision of estimating changes in mean burdens, the power of different statistical procedures and the usefulness of predictions made by mathematical modelling. In particular, identifying the most appropriate distribution allows us to account properly for genetic differences among hosts and to predict correctly the response to selective breeding.

If the infective stages of nematodes are uniformly distributed across the host range, if infection is due to a random encounter between host and parasite

and if there are no differences among grazing lambs, then the distribution of parasites among hosts should follow a Poisson distribution. The variance of a Poisson distribution is equal to its mean. However, for most parasite distributions the variance is greater than the mean (Shaw and Dobson, 1995); parasites are aggregated among their hosts or, equivalently, the distribution of parasites is over-dispersed. This is because most individuals harbour relatively small numbers of parasites, but a relatively small proportion of individuals carry most of the parasites that exist in the host population.

Ever since the pioneering work with nematode egg counts in Scottish sheep (Hunter and Quenouille, 1952) and head lice in Indian prisoners (Bliss and Fisher, 1953), the negative binomial has been widely used to provide a mathematical description of parasite distributions. The negative binomial is defined by two parameters: the mean and k (an inverse index of over-dispersion). This distribution has also been used to describe several indirect indicators of infection. For example, mean egg counts in a flock of naturally infected Scottish sheep ranged from 50 to 570 eggs per g over the grazing season and k ranged from 0.09 to 2.59 (Stear *et al*., 1995a). Generalized linear models provide a convenient way to analyse data that follow a negative binomial distribution (McCullagh and Nelder, 1989).

For many helminth infections, not all individuals are exposed to infection. Individuals may differ in their behaviours, parasites may be rare or they may show spatial or temporal variation in their distributions. In these circumstances, the distribution of parasites among hosts may be a mixture of distributions. There could be a uniform distribution of zero in unexposed individuals and an over-dispersed distribution in exposed hosts. The appropriate distribution here is a zero-inflated negative binomial.

Traditional methods, such as maximum likelihood, appear less effective in identifying and handling zero-inflated distributions than Bayesian Monte Carlo Markov Chain (MCMC) procedures (Denwood *et al*., 2008). For example, Bayesian MCMC analyses indicated that the distribution of *Nematodirus battus* faecal egg counts in Scottish lambs was zero-inflated, with the extent of zero inflation ranging from 2 to 86%, i.e. in one study, 86% of the lambs had zero egg counts as a consequence of lack of exposure. Distinguishing among individuals with zero values due to lack of exposure and individuals with zero values due to their response to infection will require more sophisticated methods of analysis (Sanchez *et al*., 2002; Churcher *et al*., 2005).

The identification of resistant animals

Currently, faecal egg counts following natural or deliberate infection are the major method used to identify resistant animals, although antibody concentrations are used in New Zealand and red blood cell concentrations (packed cell volumes) are used with *Haemonchus* infections to supplement the information provided. Other methods show considerable promise in supplementing or replacing faecal egg counts. These options include both indicator traits and genetic markers. Indicator traits include parasite-specific antibody responses (Douch *et al*., 1994), especially

IgA (Strain *et al*., 2002; Henderson and Stear, 2006) and IgE activity (Shaw *et al*., 1998, 1999), eosinophilia (Henderson and Stear, 2006), fructosamine concentrations (Heath and Connan, 1991; Stear *et al*., 2001b) and pepsinogenaemia (Berghen *et al*., 1993; Stear *et al*., 1999c). Genetic markers have recently been reviewed (Dominik, 2005; Sayers and Sweeney, 2005; Stear *et al*., 2007a). Two regions have been consistently associated with resistance to nematodes in sheep: the major histocompatibility complex on chromosome 20 (Schwaiger *et al*., 1995; Buitkamp *et al*., 1996; Stear *et al*., 1996, 2005, 2007b; Sayers *et al*., 2005; Davies *et al*., 2006; Keane *et al*., 2007) and the interferon gamma locus on chromosome 3 (Crawford and McEwan, 1999; Coltman *et al*., 2001; Davies *et al*., 2006). However, the causative mutations have still to be identified. Future research to refine these markers and to combine the information contributed by different markers is ongoing.

A different approach has been trialled in some New Zealand studies. Resilience (the ability to withstand the effects of parasitic infection) is arguably more important than resistance (the ability to control the survival of parasites). Selective breeding for resilience rather than resistance is therefore appealing (Bisset and Morris, 1996; Bisset *et al*., 1996, 2001; Morris *et al*., 2000b). To a large extent, this argument has been won. All commercial selection schemes should include production traits in the selection index, and are therefore selecting for resilience as well as resistance.

Constraints on the development of protective immune responses

The immune response to an invading parasite is complex and involves a wide variety of cells and molecules (Janeway *et al*., 2001). Immunological, ecological and genetic theories agree that the immune system protects most individuals against most potentially pathogenic organisms, most of the time. There is considerable research in defining the immune response in livestock and experimental rodents against different nematodes (Stear *et al*., 1995b; Donaldson *et al*., 1996; Gasbarre, 1997; Grencis, 2001; Gasbarre *et al*., 2001; Hein *et al*., 2001; Harrison *et al*., 2003; Shaw *et al*., 2003; Pernthaner *et al*., 2005; Nisbet *et al*., 2008; Cruikshank *et al*., 2009). However, our purpose here is not to review this research but to consider the occasional failure of the immune system to prevent disease.

Many immunologists believe that the failure of the immune system to protect against infection is a consequence of factors outside the immune system that prevent it from functioning effectively, such as protein-energy malnutrition, pathogen-induced immunosuppression or immune evasion.

In contrast, many ecologists assume that immune responses are energetically demanding (Read and Allen, 2000; Rolff and Siva-Jothy, 2003). Past evolutionary pressures have probably shaped both the variety and intensity of existing immune responses. Mammals have evolved under conditions where food is often scarce and only obtainable at some risk. Consequently, there has been considerable pressure to maximize energy and protein use. Under some circumstances, it may be better to gather less food and mount weaker immune

responses. In other words, maximizing overall fitness could increase the vulnerability to disease of some individuals.

In contrast, a purely genetic approach argues that variation in immune responsiveness and susceptibility to infection is at least partly a consequence of variation in immune response genes. In addition to the wide variety of immune deficiency genes that disable components of the immune response, there is also considerable polymorphism in genes that have more subtle effects on immune responsiveness. Many of these genes will be recent mutations that compromise the effectiveness of the immune response. Some of these polymorphisms may be maintained by a balance between competing effects (such as resistance to different diseases). But many and perhaps most of these variants are not (or no longer) selectively advantageous and may be in the process of being eliminated by natural selection.

These differences are important. If the immune system is operating at optimal levels for minimizing disease, then any attempt to alter the intensity or specificity of immune responses could increase disease susceptibility. However, if current levels of immunity are shaped by past mutations and evolutionary pressures, then disease resistance is unlikely to be optimal. This is especially true for humans and domestic animals, where current levels of nutrition and disease exposure differ from the evolutionary past.

Despite the positive evidence from experimental and commercial breeding schemes in Australia and New Zealand, there is considerable divergence of opinion about the wisdom of selective breeding. Ecologists are concerned that in multispecies communities, preferential removal of one parasite species, by vaccination or selective breeding, could provide a niche for a more pathogenic assemblage (Lello *et al*., 2006). Some evolutionary biologists argue that the pathogen will out-evolve the host, rendering selective breeding futile. Some laboratory studies suggest immunological trade-offs exist between different diseases (Graham, 2002). Consequently, selecting for resistance to one type of disease could decrease resistance to other types of disease, possibly by biasing the immune response to Type 1 or Type 11 T helper cell responses (Janeway *et al*., 2001).

There is little doubt that resistance to a specific disease can have costs, at least in livestock. For example, there is a genetic relationship between increased milk production and increased susceptibility to mastitis (Heringstad *et al*., 2000). There is also an unfavourable genetic correlation in sheep between increased wool production and decreased resistance to nematodes (Eady *et al*., 1998). Selective breeding for increased immune responses in pigs has also shown that resistance to some diseases is enhanced but resistance to other diseases is decreased (Mallard *et al*., 1998). However, these adverse relationships are quite weak and do not appear to outweigh the economic and welfare advantages of selective breeding for disease resistance.

The perspectives of the different disciplines have important implications for the control of disease by selective breeding. Genetic theory suggests that breeding for disease resistance is merely mimicking natural selection, while immunological theory argues that selective breeding for disease resistance runs counter to natural selection and could be disastrous. The issue is not fully settled, but there is no evidence from breeding programmes that selection for

resistance is either increasing susceptibility to other diseases (Stear *et al*., 2001a) or leading to parasite adaptation (Kemper *et al*., 2009). This is perhaps an area that would benefit from the application of additional mathematical modelling.

Integrated nematode control

Although selective breeding is clearly feasible and desirable, it is not advisable to rely solely on a genetic approach to control nematodes. Other options such as grazing management and nutritional supplementation can also be incorporated into a system of integrated nematode control (Stear *et al*., 2007a). More research is necessary to explore and quantify possible combinations; however, there will never be enough resources to explore all potential options. This is perhaps another area that would benefit from the application of additional mathematical modelling.

In conclusion, selective breeding for resistance offers a simple, effective and feasible method of reducing the adverse consequences of nematode infection. In the absence of better options, this should become the method of choice for most livestock breeders.

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14 Ticks and Tick-borne Diseases in Cattle

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Summary

Ticks and tick-borne diseases are of global importance, affecting livestock, human and companion animals. In particular, cattle ticks are responsible for severe economic losses in the tropics, with costs associated with parasite control along with losses in fertility, body weight and milk production. Ticks are also vectors of a variety of pathogens, with babesiosis, theileriosis and anaplasmosis being important diseases transmitted by ticks. While chemical control with acaricides has been a main tick control strategy, inadvertent selection of acaricide-resistant tick strains, which evolve faster than development of new chemicals, is a major cause for concern. Thus, exploiting host genetic resistance has a great potential to complement other control strategies and reduce the need for chemical control. Considerable variation both between and within breeds has been observed for tick resistance, with well-documented evidence of greater resistance of *Bos indicus* breeds compared with *Bos taurus* breeds. Tick load is affected by several genetically controlled morphological traits, such as coat colour and hair type, as well as genes related to the immune response. Within-breed heritability estimates vary from very low to high, depending on the evaluation method and the extent of parasite challenge, with mean values close to 0.3. Although this implies ample scope for selection, trait measurement remains difficult. Consequently, genetic markers would assist in selection under extensive farming conditions. For this purpose, quantitative trait loci (QTL) studies have been undertaken with results reported in this chapter. Tick-borne diseases are a more complex problem than ticks themselves, with resistance to such diseases not as well documented as resistance to ticks. In general, *B. indicus* cattle are more resistant to tick-borne diseases, with some evidence of variability in resistance to babesiosis and theileriosis. In summary, breeding for genetic resistance is potentially a promising means to control ticks, although the same cannot yet be stated for tick-borne diseases. Large efforts are currently being made in Australia and Brazil to develop genetic markers for tick resistance and to develop tick vaccines.

Introduction

Ticks and tick-borne diseases are of global importance because of their economic and health implications in livestock, human and companion animals (Jongejan and Uilenberg, 2004). An earlier study estimated the global cost of ticks and tick-borne disease control to be US$7 billion (McCosker, 1979). Since then there have been several reports on economic costs of specific tick-borne diseases indicating that the earlier report is an underestimate (Jongejan and Uilenberg, 2004). Cattle ticks are responsible for severe economic losses in both dairy and beef cattle enterprises in the tropics (Jonsson, 2006). The main economic impacts of ticks are the costs involved with parasite control along with losses in fertility, body weight and milk production.

The direct effects of tick parasitism are weight loss, anaemia as well as secondary infections in the parasite fixation site. Cattle tick (*Rhipicephalus* (*Boophilus*) *microplus*) has a preferential distribution in areas with high temperature and humidity, and is widely spread in the tropics. Although it is difficult to quantify the economic losses related to tick parasitism, there are some estimates available in the literature. Losses with tick infestations in Brazil were estimated to be on the order of US$800 million/year (Martinez *et al*., 2006). Furlong *et al*. (1996) estimated a 23% reduction in milk production in cross-bred Gyr (*Bos indicus*) × Holstein (*Bos taurus*) cows, which are considered to be moderately resistant. In cross-bred *B. taurus* × *B. indicus* beef cattle, Bianchini *et al*. (2007) estimated that treating animals with acaricides would increase their average final weight gain by 13 kg, compared with animals that received only anthelmintic treatment. The annual global costs associated with ticks and the diseases that they transmit to cattle amounted to US$13.9–18.7 billion (de Castro, 1997). In Australia, cattle tick is distributed around the northern coastal areas as far south as the Queensland–New South Wales border, where it is held by a quarantine boundary at an annual cost of *c*.AU$7 million (White *et al*., 2003).

Ticks are also vectors of a variety of pathogens that are implicated in severe pathologies in many mammalian species (De La Fuente *et al*., 2008). In cattle, babesiosis, theileriosis and anaplasmosis are some of the important diseases transmitted by ticks. Recovered animals normally remain tolerant, especially where these diseases are endemic (endemic stability). However, these diseases impair cattle production in developing countries and are especially harmful in areas where ticks are not present during all seasons of the year (enzootic instability).

Chemical control has been the main strategy to overcome tick infestations, but costs associated with treatment are considerable. In addition to the price of chemicals, indirect costs include reduced feed intake due to individual sensitivity to acaricides and from animal management factors. Animal stress from management is particularly critical in extensive production systems, in which chemical control sometimes involves moving animals across long distances to dipping facilities (Jonsson, 2006). Still, the most critical concern with the use of acaricides in tick control is the selection of chemical-resistant tick strains, which evolve faster than the development of new chemicals for tick control.

For example, there were concerns that strains of cattle ticks in Queensland developed resistance to all of the acaricides used for cleansing cattle before transportation (Kemp *et al*., 1999). Other concerns are the impacts on environment and human health due to the presence of chemical residues in animal food products.

Vaccines have also been proposed for tick control. The first commercial recombinant vaccine (Bm86) against *R. microplus* was released in 1995 (Willadsen *et al*., 1995). Even though this vaccine had a desired effect in reducing the number of engorging female ticks and controlling their reproductive capacity, it was widely recognized that it had relatively small impact on the practical control of ticks owing to scientific and commercial reasons (Willadsen, 2006). While the idea of biological control with natural predators of ticks such as ants, mites, birds, parasitoid wasps, fungi and nematodes is also appealing and showed some promise (for detailed review, see Samish and Rehacek, 1999; Jonsson and Piper, 2007), issues such as manufacture and stability of the living agents in the field need to be resolved before it can be applied on a large scale (Willadsen, 2006). Given this scenario, exploiting host genetic resistance is an alternative that, in combination with other tick control strategies, has a great potential to reduce the need for chemical control. In this chapter, an attempt is made to summarize the current state of knowledge with regard to host resistance to ticks and tick-borne diseases in the tropical world, with special emphasis on its implications on cattle farming.

Host Resistance to Ticks

Behavioural, morphological, physiological and environmental aspects of tick resistance

Tick load is known to be affected by several morphological traits, most of which have a highly inherited pattern, such as coat colour, hair type, hair thickness and hair length. Physiological status, such as age and pregnancy, is also a determinant of the level of resistance throughout the lifetime, as well as environmental factors, mainly temperature and humidity, that affect the exposure to the parasite as well as parasite survival in the host. Grooming behaviour may also play an important role in the success of a host in eliminating parasites.

Compared with cohorts, darker-coloured animals are more susceptible to ticks than lighter-coloured ones (Fraga *et al*., 2003; Gasparin *et al*., 2007), females are more resistant than males, pregnant cows are less resistant than non-pregnant ones and younger animals are more resistant than older ones (Utech *et al*., 1978; Silva *et al*., 2010).

A relationship between hair phenotype and tick susceptibility has been observed in several experimental datasets. In a natural infestation survey of *c*.700 Caracu cows, a Brazilian creole *B. taurus* breed, Fraga *et al*. (2003) reported a greater number of engorged parasites in animals with thicker hair. The association between hair morphology and number of ticks was also described in a Holstein × Gyr F_2 generation population in Brazil, in which

animals with long, curly hair had twice more ticks than those with short, straight hair (Gasparin *et al*., 2007).

Madalena *et al*. (1985) examined causes of variation in tick burdens under natural infestation, and found slightly higher correlations between counts in the same animals when both counts were made in spring/summer ($r = 0.40$) or in autumn/winter ($r = 0.39$), compared with counts made in different seasons ($r = 0.24$). Level of infestation affected these correlations, with the maximum value at around the mean tick burden (185 ticks per animal). When the levels of infestation were too low or too high the correlations between counts decreased.

Immunological aspects of resistance to ticks

Ticks and tick-borne pathogens engage in complex interactions with their mammalian hosts, involving interplay between the defensive responses and counter-responses of host, tick and pathogen. These complex interactions are being unravelled by the advances in genomics leading to the novel means of parasite control targeting the tick–host–pathogen triangle (Jongejan *et al*., 2007).

To succeed in their life cycle, ticks need to spend a long period attached to the host, ranging from several days to weeks. Blood feeding during this period stimulates haemostatic, inflammatory and immunological responses in the host in an attempt to eliminate the parasites or to impair parasite development. Both regulatory and effector pathways of the host immune response are stimulated, and it has been demonstrated that consecutive infestations result in increased resistance (Wagland, 1978). Despite that, immunity to one species of tick does not assure protection against other species, as demonstrated by Miranpuri (1989) in cross-bred cattle infested by *R. microplus* and *Hyalomma anatolicum anatolicum*.

To understand the host response to ticks one needs to consider that tick saliva has evolved to produce a mixture of molecules that act as immune modulators, destroying tissue integrity and blocking host haemostatic cascades (Singh and Girschick, 2003; Brossard and Wikel, 2004; Ribeiro *et al*., 2006). In general, tick salivary gland extract (SGE) downregulates the immune system. This effect is well described in many tick–host models and it is expected to be a relatively long-lasting effect, since it has been demonstrated that SGE is present in the skin for up to 5 days after larvae infestation (Allen *et al*., 1979). Among the targets of SGE immune modulators are the dendritic cells. Maturation of these cells is impaired when they are exposed *in vitro* to *Ixodes ricinus* saliva. *In vivo*, dendritic cell migration from the bite site on the skin to draining lymph nodes is reduced (Skallova *et al*., 2008).

Genetic variation for tick resistance results from differences in a variety of mechanisms that are not fully understood, yet involvement of genes related to the immune system in many of these mechanisms is undeniable. Therefore, comparison of the immune profile in resistant versus susceptible breeds has been the major strategy to elect candidate mechanisms to explain resistance. For instance, acquired resistance is developed earlier and reaches a higher level in resistant

than in susceptible breeds (Wagland, 1978). Although the exact mechanisms that led to this difference are not clear, it is possible that they may include evading the inhibitory effects of tick saliva on the host's immune response.

For example, Turni *et al*. (2002) demonstrated that lymphocytes from resistant *B. indicus* were less affected *in vitro* by the inhibitory effect of tick SGE than lymphocytes from susceptible *B. taurus*. Kashino *et al*. (2005) reported higher levels of IgG1 and IgG2 in Nelore animals heavily exposed to ticks, compared to susceptible Holstein. However, differences in IgG levels resulted mainly from a pronounced suppression of anti-tick saliva IgG antibody responses in Holstein rather than from an increase in IgG secretion in Nelore. Acute-phase proteins, components of innate immune responses, are also implicated in differences between susceptible and resistant breeds of cattle (Carvalho *et al*., 2008).

In some instances downregulation of the immune response seems to be advantageous for the host. When cytokine mRNA levels were examined at the extremes of tick resistance in four genetic groups, downregulation of interleukin 8 (IL8) was observed at the resistant extreme of the cross-bred 50% Angus, 50% Nelore group (Regitano *et al*., 2008). IL8 is a leukocyte chemoattractant protein that controls movement of leukocytes to the infection site, besides acting in the stimulation of angiogenesis. The relative advantage of inhibiting IL8 for the host's defence is unclear. It is curious that this cytokine is inhibited by the salivary extract of several tick species (Hajnická *et al*., 2001), which leads one to consider that the inhibition would be beneficial to the parasite. Interestingly, *IL8* downregulation was observed only in the Angus × Nelore cross. Among the crosses studied, this was the only one sired by a British breed in that study. This finding suggests that specific mechanisms of host–parasite interactions were perpetuated in some breeds and not in others, possibly as a result of genetic drift during the establishment of breeds.

Other evidence of this breed-specific pattern of host–parasite interaction was given by Piper *et al*. (2008), who found differences between expression profiles of skin collected from the tick attachment site versus non-attachment sites in Holstein but not in Brahman. Field observations also support the idea that a variety of host mechanisms must be involved in blocking the parasite cycle, since some resistant individuals within the same experimental group develop a severe cutaneous reaction while others do not. Some have a great number of undeveloped nymphs attached to the skin while others have no parasites of any stage.

Between- and within-breed variability for tick resistance

Despite the complexity of tick–host interaction, there is a genetic component of variation in host resistance to ticks. This genetic variation is expected to be under the control of multiple genes if we consider all the mechanisms involved in resistance that vary from inherited differences in morphological traits to genetically determined variation in the immunological response to parasites, as discussed in previous sections.

The clearest difference in tick resistance among cattle breeds is the one between *B. taurus* and *B. indicus*, with the latter being several times more resistant (Lemos *et al*., 1985). In Brazil, higher resistance in *B. indicus* compared with *B. taurus* was first reported by Villares (1941). Later, Lemos *et al*. (1985) found an exponential relationship between tick burdens under heavy natural infestations and the Holstein–Friesian proportion in crosses with the Guzerat breed (*B. indicus*). Comparisons between sprayed and non-sprayed artificially infested cows revealed a 25% reduction in milk yield of mature, non-sprayed Holstein–Friesian cows, while no difference in yield was observed in infested versus sprayed F1 or 5/8 Holstein–Friesian × Guzerat crosses (Teodoro *et al*.,1998). The effect of *B. indicus* genotype on resistance is consistent among different crosses. For example, under relatively mild natural challenge, Teodoro *et al*. (1994) did not find differences in tick burdens among cross-bred progeny of Holstein, Jersey and Brown Swiss sires at the same *B. indicus* gene fraction.

In tropical Australia, the use of tick-resistant *B. indicus* cattle and their crosses has been widely practised over the years. Utech *et al*. (1978) reported a comprehensive study assessing tick resistance as determined by the percentage of larval ticks that failed to survive maturity following artificial infestations with tick larvae in various breeds of beef and dairy cattle. In beef cattle, not surprisingly, Brahmans were found to be more resistant than British cattle. In dairy breeds, *B. taurus* Jersey cattle were reported to be more resistant than Guernsey, Australian Illawara, Shorthorn and Friesian but not significantly different from cross-bred Australian Milking Zebu.

Recently, Prayaga (2003) reported breed group differences with significantly ($P < 0.05$) lower tick counts in a Zebu-derived breed group (predominantly Brahmans) compared with Sanga-derived (Belmont Red–Africander × Hereford–Shorthorn cross) and a tropically adapted British breed group (Belmont Adaptaur × Hereford–Shorthorn cross). In this Australian cross-breeding study, it was reported that as the Zebu proportion in the cross increased, the number of engorged ticks decreased. Further, Frisch and O'Neill (1998) also reported that Brahmans record slightly decreased growth rate when untreated for ticks and worms, but they still exceed the growth rate of British breeds under low to moderate natural parasite challenge in tropical climates of northern Australia. This further supports the genetic advantage of tropically adapted *B. indicus* breeds and their crosses over *B. taurus* breeds. Prayaga (2003) also reported a significantly negative (favourable) breed additive component of the Zebu breed for tick counts, suggesting their genetic resistance. A significantly negative (favourable) direct dominance effect between taurine × indicine breed crosses further supported the significant heterosis (–40%) reported in crosses involving Zebu and British breed group crosses in this study.

Genetic variation is also seen within a breed. Heritability estimates varied from very low to high (Alencar *et al*., 2005). This broad variation is attributed to both evaluation method (artificial × natural challenge) and additive genetic variation for resistance, which is intrinsic to each population studied (Table 14.1). Further, differences in heritability also reflect the extent of natural parasite challenge under extensive conditions, enabling the expression of variation in tick resistance across various studies. Artificial challenge is more

Table 14.1. Estimates of heritabilities of tick infestation from the literature.

Reference	Location	Breed[a]	Trait	Challenge	h^2 (s.e.)[b]
Wharton *et al.* (1970)	Australia	Shorthorn	Count	Natural and artificial	0.39
Madalena *et al.* (1985)	Brazil	Cross-bred	Count	Artificial (sires)/ natural (progeny)	0.20 (0.06)
Mackinnon *et al.* (1991)	Australia	AX, AXBX	Count	Natural	0.34 (0.05)
Burrow (2001)	Australia	AX, AXBX	Count	Natural	0.44
Henshall *et al.* (2001)	Australia	AX, AXBX	Count	Natural	0.42
Fraga *et al.* (2003)	Brazil	Caracu	Count	Natural	0.22
Fraga *et al.* (2003)	Brazil	Caracu	Score	Natural	0.15
Henshall (2004)	Australia	HS	Count	Natural	0.44
Prayaga and Henshall (2005)	Australia	Cross-bred	Count	Natural	0.13 (0.03)
Peixoto *et al.* (2008)	Brazil	Holstein × Gir	Count	Artificial	0.21 (0.12)
Prayaga *et al.* (2009)	Australia	Brahman	Score	Natural	0.15 (0.10)

[a]HS–F_{5+}, Hereford–Shorthorn (*B. taurus*) cross; BX–F_{5+}; Brahman (*B. indicus*) × HS; AX–F_{5+}, Africander (Sanga, a tropically adapted taurine breed) × HS, F_{3+}; AXBX, a cross of AX and BX. [b]Standard error, whenever available.

efficient in controlling the environment variance, since during the free-living stage of a tick's life cycle it is very sensitive to temperature, humidity and species of grass and, as pointed by Madalena *et al*. (1985), the correlations between counts in the same animal may be low if counts are made in different seasons. A mean of 0.34 ± 0.06 for the estimated heritability across breeds was reported in a review by Davis (1993). Despite the generally moderate heritability estimates across various breeds implying a scope for selection, one difficulty lies with the trait measurement hindering application in traditional genetic evaluation systems.

Breeding for Host Resistance to Ticks

Exploring genetic resistance in breeding programmes

Selecting for increased host resistance is a low-cost and highly effective way of addressing the economic aspects of tick control. There have been several strategies to achieve this under field conditions. One of the effective ways to tackle the problem of cattle ticks has been the use of breeds or strains of cattle

that are relatively resistant, for example Brahmans in northern Australia. Further, cross-breeding has also been effective in introgressing desirable resistance attributes into a susceptible breed.

However, it is important to be mindful of the correlated responses in other economic traits such as growth, meat quality or milk yield as a consequence of selection for tick resistance. In Australia, several studies have reported low and non-significant genetic correlations between tick counts and various productive, adaptive and pubertal traits (Davis, 1993; Prayaga *et al*., 2009), indicating that the selection for tick resistance may not have any deleterious effects on other economic traits. However, caution needs to be exercised in selection programmes, particularly those using genetic markers, and research needs to be continued to screen for any associated effects on other economic traits.

In an attempt to develop a tick-resistant British line of cattle, a Hereford–Shorthorn line (Belmont Adaptaur) was selected in Australia for tick resistance over several generations (Frisch *et al*., 2000). It was reported that a genetic trend of a linear reduction in tick count, at the rate of 7 ticks/year, was achieved, reducing tick counts from a mean of 275 ticks/animal/day in 1983 to about 40 ticks/animal/day in 1998. Owing to this selection, a correlated response in weight (1.28 kg/year at 18 months of age) was achieved in this experiment and the authors argued that this increased weight was due to genetic improvement in tick resistance as no direct selection was applied for growth potential. Prayaga and Henshall (2005) reported low and non-significant genetic correlations between growth traits and tick counts, suggesting that selecting for growth under tropical conditions is unlikely to improve tick resistance at a genetic level. This was observed despite the fact that there was a significant increase in post-weaning live weight gain in animals treated for ticks and worms compared with the untreated control group (Prayaga, 2003).

In Brazil, evaluation of tick resistance was applied to a conventional progeny testing programme of dairy *B. taurus* × *B. indicus* sires. Tick resistance was scored after artificial infestation in young bulls, before semen collection. In addition, progeny were scored for tick burdens under natural infestation (Madalena, 1999). Culling the 10% most infested Holstein–Friesian heifers was predicted to reduce total infestation by 18%. This reduction increased to 26% when *B. indicus* represented three-fourths of the cross with Holstein–Friesian heifers. However, a decrease in correlation between counts of the same animal across time could lead to lesser benefits. Heritability and repeatability of log count of ticks per animal were 0.49 and 0.62, respectively. Genetic correlations of this trait with milk, protein and fat yields were low (0.06, −0.14 and 0.07, respectively).

Commercial expected progeny differences (EPDs) for tick burdens are currently estimated and utilized in Braford and Brangus (5/8 Aberdeen Angus, 3/8 Nelore) breeding programmes in Brazil (http://www.gensys.com.br/). The evaluations are conducted under natural infestation by the methodology described in Cardoso *et al*. (2006), in which tick counts are made in only one region of the body, the inner hind legs.

Finding genes and markers for tick resistance

Genetic variation in tick resistance both within and across breeds should, in principle, enable successful implementation of selection programmes. However, recording tick counts is not economical and not even feasible under extensive farming systems, highlighting the importance of developing genetic markers. While some pathways involved in resistance to cattle tick are known, little is known on how variation at specific loci contributes to the degree of resistance. Several efforts have been made in an attempt to isolate and validate markers for the selection of resistant genotypes, and these will be discussed in this section.

The first reports on markers for tick resistance were studies on blood protein polymorphisms (Francis and Ashton, 1967; Ashton *et al*., 1968; Panepucci *et al*., 1989). In all the studies, the serum amylase locus was associated with tick number. Curiously, few other investigations on this association were reported afterwards.

Frisch (1994) reported a Hereford–Shorthorn line of cattle selected for tick resistance under tropical conditions in Australia to be apparently segregating for a major gene for tick resistance. However, later studies found little support for a major gene, indicating that resistance attributes are more likely to be due to polygenic effects (Henshall, 2004). The obvious candidate genes for association with tick resistance are the genes for immune modulators and effectors. Associations between bovine major histocompatibility complex (MHC) (BoLA) class II alleles and tick resistance have been reported (Stear *et al*., 1990; Acosta-Rodríguez *et al*., 2005). An association between BoLA alleles DRB3.2 *18, *20 and *27 and lower tick number was found in a reference Holstein × Gyr F_2 population in Brazil (Martinez *et al*., 2006). In a study of natural infestation, genotypes for a microsatellite marker close to the IL4 locus were associated with tick number in cross-bred *B. taurus* × *B. indicus* and in pure *B. indicus* (Regitano *et al*., 2008).

Genomic scans for quantitative trait loci (QTL) controlling tick resistance are presently being undertaken in a reference F_2 family in Brazil. In this project a Holstein × Gyr population of 382 F_2 animals was developed from 1999 to 2005. The F_2 generation was evaluated for tick resistance by experimental challenge during two seasons, as described in Gasparin *et al*. (2007). QTL detected to date have been dependent on the season when the parasite load was scored, which could be interpreted as a QTL–environment interaction. A total of five QTL were mapped for the trait scored during the rainy season and three for the same trait scored in the dry season (Regitano *et al*., 2008). More recently, two additional QTL were detected in this population, explaining 7.1% of the phenotypic variability observed during the dry season (Peixoto *et al*., 2008). In total, the QTL mapped in this resource population explained 13.1% of the phenotypic variation during the rainy season and 18.4% of the phenotypic variation in the dry season (Table 14.2). Half of the QTL mapped in that resource population deviate from the pure additive genetic model, which is in agreement with the heterosis found in crosses between *B. taurus* and *B. indicus*. It is also in agreement with the observed dominant behaviour of tick resistance in those crosses.

Table 14.2. QTL for tick resistance identified in a Holstein × Gyr F_2 population.

Season[a]	Chromosome	*P*[b]	Position (cM)	D[c]	% Variance explained[d]
Dry	7	0.01	73	Yes	1.90
	10	0.01	18	No	6.20
	14	0.05	25	Yes	3.20
	15	0.01	60	No	2.26
	27	0.05	5	No	4.80
Rainy	4	0.05	98	Yes	2.4
	5	0.05	132	Yes	1.70
	11	0.05	32	No	3.4
	18	0.01	60	Yes	1.97
	23[e]	0.05	50	No	3.6

[a]Season in which ticks were counted; [b]F statistics' *P* value; [c]dominance deviation; [d]proportion of the phenotypic variance explained by the QTL; [e]this QTL was not tested for dry-season data.

With the newly available bovine SNP chips, high-density SNP genotyping is the most promising strategy for fine-mapping these QTL and for functional SNP (quantitative trait nucleotide (QTN)) detection. Once detected, these tick resistance QTNs would need to be validated for use in marker-assisted selection in other populations. The progress being made in this regard is evident from a recent report of an SNP assay for detecting tick resistance in cattle (Barendse, 2007). This study reported several genes from the immune system as being linked to tick resistance, although the MHC was not one of them.

Results from gene expression profiles obtained after a challenge of resistant versus susceptible animals are a good source of candidate genes for tick resistance. Recent work by Wang *et al*. (2007) described at least 66 genes with differential expression in tick-challenged skin of resistant versus susceptible *B. taurus*. Among these, Type I, III and V collagen genes had higher expression in resistant animals than in susceptible ones, and keratin genes were more suppressed after challenge in susceptible animals than in resistant ones. These results suggest that some of the genetic variation for tick resistance may be explained by genes related to skin structure.

Host Resistance to Tick-borne Diseases

Tick-borne diseases are a more complex problem than ticks themselves. There are a number of different pathogens that may be transmitted by each tick species, and the incidence of tick-borne diseases is dependent on seasonal and geographical distribution of the vector. As with most vector-dependent diseases, outbreaks are expected after long periods of low exposure to the vector or introduction of an infected vector to a vector-free area.

In bovine species, the most relevant pathogens are *Babesia bovis*, *Babesia bigemina* and *Anaplasma marginale*, transmitted by *R. microplus*; *Babesia*

divergens, transmitted by *Ixodes ricinus*; *Theileria annulata*, transmitted by ticks from the genus *Hyalomma*; and *Theileria parva*, transmitted by *Rhipicephalus appendiculatus*, *Rhipicephalus zembeziensis* and *Rhipicephalus duttoni* (Table 14.3). Given the variety of tick-borne pathogens, resistance will be considered for each pathogen separately.

Table 14.3. Tick-borne pathogens that affect cattle. (From Maillard *et al*., 2003; Shkap *et al*., 2007; De La Fuente *et al*., 2008.)

Pathogen	Major vector(s)	Disease	Known geographic distribution	Host(s)
Babesia bigemina	*Rhipicephalus* spp.	Cattle babesiosis	Africa, America, Asia, Australia	Cattle, buffalo
Babesia bovis	*Rhipicephalus* spp.	Cattle babesiosis	Africa, America, Asia, Australia	Cattle, buffalo
Babesia major	*Haemaphysalis* spp.	Cattle babesiosis	Europe	Cattle
Babesia divergens	*Ixodes* spp.	Cattle babesiosis	North-west Europe, Spain, UK, Ireland, Tunisia	Cattle, human
Theileria annulata	*Hyalomma* spp.	Tropical theileriosis	Eurasia, Africa, Central Asia	Cattle, camel
Theileria parva	*Rhipicephalus appendiculatus*	East Coast Fever	Africa	Cattle
Theileria lawrencei	*Rhipicephalus zambeziensis*	Corridor disease	Africa	Cattle
Theileria mutans	*Amblyomma hebraeum, A. lepidum, Amblyomma variegatum, Amblyomma cohaerens, Amblyomma gemma*	Benign theileriosis	Africa	Cattle
Theileria taurotragi	*R. appendiculatus, Rhipicephalus pulchellus, R. zambeziensis*	Benign theileriosis	Africa	Cattle
Ehrlichia ruminantium	*Amblyomma* spp.	Heartwater	Sub-Saharan Africa, Carribean Isalnds	Cattle, sheep, goats
Dermatophilus congolensis	*A. variegatum*	Dermatophilosis		Cattle
Anaplasma marginale, Anaplasma centrale	Various (arthropod, mechanical, e.g. needles)	Anaplasmosis	Worldwide	Cattle

Immunological responses to tick-borne diseases

Cattle infected with *B. bovis* develop fever, depression and haemoglobinuria, often accompanied by anaemia and abortion. Neurological signs are frequently observed and a fatal cerebral disease, associated with the adherence of infected erythrocytes to brain microcapillary endothelial cells, may also occur. Infection with *B. bigemina* is usually less severe, with less pronounced fever and sporadic neurological signs (Brown, 2001; Jonsson *et al*., 2008).

Immunity to the genus *Babesia* is documented in several mammalian species, and efforts to understand protective mechanisms and immune-dominant proteins have been made in order to develop vaccines. A peculiarity of *Babesia* parasites is that they replicate exclusively within the erythrocytes, which is devoid of molecules from the MHC.

Protection against *Babesia* infection involves both innate and acquired immunity. In naive animals infected with virulent *B. bovis* parasites, resolution of acute infection results from the innate immune response (Brown, 2001). This response depends on activation of macrophages via interferon gamma (IFN-γ) and parasite-derived products, such as deoxyribonucleic acid (DNA), followed by production of toxic macrophage metabolites, including nitric oxide (NO). The spleen is the clearance site for *B. bovis*-infected erythrocytes, with splenectomized animals developing clinical disease earlier than non-splenectomized ones (Zintl *et al*., 2005; Brown *et al*., 2006). This spleen-dependent innate response seems to be more effective in young than in adult bovines, which could be related to faster activation of IL-12- and IFN-γ-mediated NO production in spleen cells (Goff *et al*., 2001).

Regulation of innate immunity is crucial. Some immune mediators produced by cells of the innate immune system that are protective against intracellular pathogens, such as IFN-γ, type I IFN, NO and tumour necrosis factor alpha (TNF-α), are also implicated in the severe pathogenesis associated with infection by *B. bovis* and *Theileria* spp. parasites (Brown, 2001; Brown *et al*., 2006).

Acquired immunity to *Babesia* spp. may be developed after immunization or persistent infection. The protective role of antibodies is demonstrated by passive protection obtained *in vivo* by immune serum administration. However, there is no evidence of the effect of immune serum on parasite viability *in vitro*, suggesting that these antibodies act as opsonins for activated macrophages, instead of as neutralizing antibodies (Brown, 2001).

Recovery from acute infection with *B. bigemina* or *B. bovis* does not result in cure. Instead, recovered animals remain persistently infected and do not develop clinical disease upon reinfection with the same strain. These healthy recovered animals act as reservoirs for the maintenance of vector infection rates. In tropical areas, vector and reservoir availability throughout the year guarantees exposure of young animals to *Babesia* spp. before the passive immunity acquired trough colostrum is lost, i.e. when calves are approximately 2 months old. This equilibrium between exposure and immune response results in an endemic stability. If this equilibrium is broken by long periods of low exposure to the infected vector, clinical disease may occur (Oliveira *et al*., 2008).

However, based on serological evidence in Australia, it was reported that the endemic stability is uncommon with respect to the three tick fever organisms, *B. bovis*, *B. bigemina* and *A. marginale* (Serugga *et al*., 2003).

Theileriosis is a severe disease of cattle and domestic buffaloes caused by protozoan of the genus *Theileria*. *T. parva*, causing East Coast fever (ECF), is essentially present in central and eastern Africa. *T. annulata*, causing tropical theileriosis or Mediterranean Coast fever (MCF), is present in northern Africa, southern Europe, the Middle East and central Asia. Both diseases are characterized by lymph node augmentation, high fever, anorexia, nasal and ocular discharge and diarrhoea. Death occurs after 7–10 days in 90% of cases.

As with most members of the phylum *Apicomplexa*, *Theileria* infect nucleated cells, macrophages and lymphocytes, before developing to a merozoite stage in the erythrocytes. During this period of infection, they hide within these cells and modulate host gene expression to allow parasite replication.

Macrophages infected by *T. annulata* are upregulated for pro-inflammatory cytokines, IL-1β, IL-6, TNF-α and BoLA class II genes, and downregulated for surface proteins, most of which are involved in cell signalling. Some of the clinical signs of the disease may be related to the parasite-induced cytokine profile, since they are similar to administration of IL-6 and TNF-α. A detailed review on theileriosis-related gene expression profiles is found in Glass and Jensen (2007).

Parasite dose and virulence are determinants of disease severity, which may go from acute lethal to clinical recovery. In the latter, persistent and solid immunity is achieved (Preston *et al*., 1999).

Genetic variation for resistance to tick-borne diseases in cattle

Host resistance to diseases transmitted by ticks is not as well documented in cattle as is resistance to ticks themselves. In general, *B. indicus* cattle are more resistant to tick-borne diseases, although this resistance does not apply to *A. marginale* (Bock *et al*., 1997, 1999).

In the case of *Babesia* spp., infection rates are affected by age, climate, soil and host genetics. While the *B. indicus* Nelore breed is considered resistant to *B. bigemina*, the frequency of infection in this breed is similar to cross-bred and *B. taurus* animals (Oliveira *et al*., 2008). So the main difference among resistant and susceptible breeds is not related to whether they become infected but, rather, to how they overcome babesiosis, i.e. resilience. By comparing ticks collected from pure Nelore and cross-breeds, Oliveira *et al*. (2008) demonstrated that there is no difference in tick infestation rate among genetic groups. The practical implication of this finding is that introduction of *B. indicus* would not result in clearing the vector, i.e. reduce infection levels, and would not break endemic stability.

Within-breed individual variability occurs for babesiosis (Bock *et al*., 1999), but no heritability estimate is available, since measuring resistance in a large number of individuals is not possible. Three categories of resistance were

reported by Benavides and Sacco (2007) upon challenge of pure *B. taurus* naive heifers with *B. bovis*: susceptible, intermediate and resistant. The susceptible category represented 45% of the assayed animals (120 Hereford and 120 Aberdeen Angus) and was characterized by animals that had to be medicated to avoid death. The second and third categories overcame infection without treatment. Animals classified as intermediate, 27% of those challenged, had moderate reduction of packed cell volume, without clear manifestation of clinical signs. Resistant animals, the remaining 28%, had less of a packed cell volume reduction with only minor increase in body temperature. This is the only report of variation in resilience to *Babesia* in *B. taurus*. Mechanisms underlying haemoparasite resistance are not clear, but they may be related to both impairment of parasite replication and mechanisms of anaemia control (Naessens, 2006).

Genetic variation is also reported for resistance to theileriosis. The mortality rate from ECF can be up to 100% in cattle from non-endemic areas, but is usually low in indigenous zebu cattle from endemic areas. The Sahiwal (Glass *et al*., 2005) and Kenana zebu breeds are considered resistant to tropical theileriosis (*T. annulata*), as are some taurine breeds that emerged within endemic areas.

Genes and Markers for Resistance to Tick-borne Diseases

No relevant information on markers or candidate genes associated with bovine resistance to babesiosis is found in the literature, but clues on the genetic control of variation in *Babesia* resistance may come from model species. In mice, a locus linked to resistance to babesiosis was mapped near the telomeric region of mouse Chr 16, using a C57BL/6 (resistant) C3H/HeJ (susceptible) backcross and recombinant inbred strains (Aguilar-Delfin *et al*., 2003).

Some pathways and candidate genes for resistance to *Theileria* are emerging from comparisons of immune profiles of resistant and susceptible animals. Glass *et al*. (2005) compared responses to experimental infection with *T. annulata* in Sahiwal and Holstein calves. All Sahiwal calves survived without treatment and with few clinical alterations, accompanied by significantly less response in acute-phase proteins, α_1 acid glycoprotein and haptoglobin than for the Holsteins. Additionally, the Sahiwals had lower resting levels of α_1 acid glycoprotein than the Holsteins ($P < 0.05$). Production of a third acute-phase protein, serum amyloid A, had very similar kinetics in both breeds. Production of acute-phase proteins is stimulated by cytokine pathways that might also be involved in pyrexic, cachectic and anorexic signs in tropical theileriosis. From these results it is possible to postulate that the ability to avoid overstimulation of specific cytokine pathways could help in host recovery from infection.

Using the same rationale of comparing Sahiwal and Holstein, but aiming at global transcription patterns through the use of microarrays, Glass and Jensen (2007) demonstrated that a variety of cellular pathways are altered upon *in vitro* infection of monocytes with *T. annulata*. These pathways include

Toll-like receptor and mitogen-activated protein kinase. Among the differentially expressed genes between breeds, about two-thirds were differentially expressed also in resting (non-infected) cells, showing that fundamental breed differences could account for the way they respond to infection. Further exploration of these microarray data, classifying genes according to the most common biological processes in Gene Ontology, revealed differential expression of genes encoding proteins expressed in the plasma membrane or intracellular space or related to cell adhesion (Jensen *et al*., 2008).

Although there is little development on markers for tick-borne diseases in livestock, one of the most relevant results on the use of marker-assisted selection for resistance is the work by Maillard *et al*. (2003), in dermatophilosis, a severe skin disease caused by the filamentous actinomycete bacterium *Dermatophilus congolensis* in which disease severity is related to the immune modulation exerted by the tick *Amblyomma variegatum*. Eugenic selection against the BoLA-DRB3.2*09/*45 and DQB*1804 alleles, strongly associated to susceptibility to dermatophilosis, was applied to a Brahman breeding programme in Martinique. The disease incidence was reduced from 0.76 to 0.02 in 5 years of marker-assisted selection.

Conclusions

In a study on assessing the vulnerability of the Australian beef industry to impacts of the cattle tick under a climate change scenario, White *et al*. (2003) predicted significant expansions in potential geographical impacts, with increased abundance of tick populations and reductions in cattle productivity. With global warming being perceived as a certainty, its cascading effect on various facets of cattle farming, including the increased challenge of parasitic infestations such as ticks, needs to be addressed with a holistic approach.

There have been various attempts at controlling ticks and tick-borne diseases in tropical livestock. Breeding for genetic resistance is one of the promising ways to control ticks, although the same cannot be stated for tick-borne diseases as these still need more investigation to characterize the genetic variation and to depict underlying genes and markers. On the other hand, the application of marker-assisted selection would be of greater benefit in the latter case, since conventional breeding for tick-borne diseases, i.e. scoring phenotypes for resistance in a large number of animals, is almost impractical in commercial breeding schemes.

Efforts are currently continuing with large projects in Australia (e.g. projects under the Cooperative Research Centre for Beef Genetic Technologies) and Brazil (e.g. research initiatives from Embrapa, with support from the Brazilian National Research Council (CNPq)) aiming to develop genetic markers for tick resistance and to develop tick vaccines. While these efforts will eventually lead to identification of genes or genetic markers underpinning the variation in tick resistance, future research should aim to effectively use this knowledge in breeding programmes to improve tick resistance without compromising the genetic gains accrued over generations in other economic traits.

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V Metabolic and Production Diseases

15 Metabolic Diseases in Sheep and Cattle

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Summary

In this chapter the current state of knowledge on the genetic control of host resistance and susceptibility to metabolic diseases is reviewed in sheep and *Bos taurus* cattle, considering diseases such as those caused by fungal toxins, pasture bloat, mineral deficiencies and non-infectious foot problems. For the seven groups of metabolic diseases discussed here for cattle, heritabilities averaged 0.21, and for the four groups of sheep diseases discussed, heritabilities averaged 0.30. Thus it would be possible to apply selection pressure to a population to increase resistance to most of these metabolic diseases if the economic incentive was sufficient. The genetic control of some metabolic diseases has been investigated at the level of the experimental flock or herd, by breeding resistant or susceptible lines of stock, and some experimental selection lines are discussed here, with special emphasis given to resistance to fungal toxins. Of the traits described, facial eczema (FE) disease is one of the few where selection for resistance is being applied in the industry at present, and findings from more extensive studies are presented for this disease.

Introduction

In this chapter we consider some metabolic diseases of sheep and *Bos taurus* cattle, and discuss what is known about between-animal variability in the genetic control of host resistance or susceptibility. A wide variety of such diseases of grazing sheep and cattle must be guarded against, or dealt with, at the industry or individual farmer level.

Sheep and cattle may be farmed under extensive conditions on a roughage diet, or managed intensively on a high-energy diet. Although diets are generally specified with the aim of promoting production and reproduction, some unintentionally may contain undesirable contaminants or a deficiency and therefore may be deleterious to the health of some stock, leading to metabolic disease.

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One category of metabolic diseases occurs when animals are fed diets containing contaminants such as toxins produced by fungi growing on pasture or on crops, or contaminants such as toxic metabolites from endophytes within plants. The worldwide cost of damage from these toxins, for example *Fusarium*, has been reported by D'Mello *et al*. (1999). The significance of mycotoxins to the US economy alone has been estimated at US$932 million per year (range US$418 million to US$1.66 billion) (Vardon *et al*., 2000). Animal resistance to the effects of fungal contaminants is generally moderately heritable (described below), and genetic selection for resistance could be applied to herds or flocks, to improve animal health over the long term if there is sufficient economic incentive to do so. Examples of such metabolic diseases are described in this review, including facial eczema (FE), ryegrass staggers (RGS), tall fescue toxicosis and zearalenone-induced infertility.

A second category of metabolic diseases is the animal response to a legitimate component or its deficiency in the diet, leading to a metabolic problem in susceptible stock. Examples of these diseases will also be reviewed, including pasture bloat and mineral deficiencies such as hypomagnesaemia and hypocalcaemia.

Stock managers are usually aware of metabolic diseases that might be induced by diet, and the simplest solution for any such potential cases is through management, for example by moving/changing animals to another diet. Unfortunately this is often not an option under extensive grazing conditions because a high proportion of the farm grazing area may be affected. Switching to a concentrated diet is often not an option either, because of cost implications. In some cases, a genetic selection approach has been adopted and applied experimentally, in order to increase our understanding of the disease and possible solutions. One disease (FE) will be described, where selection for resistance is now being applied at industry level.

A breed effect on disease resistance or susceptibility is the most common example or overall demonstration of genetic variability in susceptibility. Sometimes breed incidence may be confounded with regional incidence but, within a region, where herds or flocks of different breeds differ consistently in disease incidence, a genetic component to disease susceptibility is suggested. The next level at which to investigate the genetic controls of resistance or susceptibility to disease is within-breed inheritance, as discussed below.

Further classes of metabolic diseases are those described by Nicholas (2005) as 'diseases from within', meaning genetic disorders caused by inborn errors. Our collective understanding of the bovine genome has now reached the stage where the causes of some diseases have each been described in terms of a mutation in a single gene. The full documentation of these can be found at http://omia.angis.org.au/, and at the time of writing (March, 2010), there are 379 'phenes' in cattle and 188 'phenes' in sheep. These include 'single-locus phenes', some of which are 'phenes characterised at the molecular level'. A review of these genes has been published by Ibeagha-Awemu *et al*. (2008).

Facial Eczema

Facial eczema is a disease caused by sporidesmin, a toxin from spores of the saprophytic fungus, *Pithomyces chartarum* (Percival and Thornton, 1958). The spores are found on many pastures in summer and autumn in the North Island of New Zealand. The disease affects cattle, sheep and deer (Smith and Towers, 2002). Annual costs of FE vary with weather conditions, but on a national basis in New Zealand they can be up to NZ$63 million in sheep (Anon., 1990), and up to NZ$120 million in dairy cattle (unpublished estimates from an industry-wide study, 2008). Genetic control methods for FE have been studied intensively in sheep and cattle, as described below. In susceptible sheep, sporidesmin causes liver and bile duct injury, with consequential deleterious effects on growth and, in serious cases, on fleece weight, reproduction and survival. In susceptible dairy cattle, there are effects on milk production (Towers and Smith, 1978) and on cow survival in serious cases (Morris *et al*., 2002). The effects of the disease are not completely reversible, but rapid regrowth of new liver tissue occurs, and carry-over effects from one FE season to the next season's production in survivors are small (Morris *et al*., 2002). Because of the extensive grazing systems used for most beef cattle in New Zealand, compared with their more intensively managed dairy counterparts, fewer cases of FE occur in beef cattle.

Various management options have been investigated or introduced to minimize the impacts of FE. They include fungicide-spraying of pasture (e.g. Campbell and Sinclair, 1968), controlled stock movement to avoid contaminated paddocks and growing specific alternative crops for grazing during the FE season. A biological control was tried by competing resident toxic *P. chartarum* strains with introduced non-toxigenic isolates, but the most successful method in use today is zinc prophylaxis in animals.

Following early observations of between-animal variation in resistance or susceptibility, genetic selection of FE-tolerant animals has been considered since the 1970s. In sheep the first heritability estimate was reported to be 0.42 ± 0.09 (Campbell *et al*., 1981), based on the liver injury scores obtained from sire progeny tests, after sporidesmin challenge. A performance test method was made possible by Towers and Stratton (1978), who demonstrated that FE-affected animals could be phenotyped or ranked using a biochemical marker, gamma glutamyltransferase (GGT), under controlled sporidesmin dosing. This enzyme leaks from damaged bile ducts and is measurable in blood. Later, a heritability estimate of 0.45 ± 0.05 was obtained for log GGT (Morris *et al*., 1995a). Making use of these developments, resistant and susceptible lines of Romney sheep were established at Ruakura in 1975, and later a control line was added. Potential replacements were selected according to degrees of resistance or susceptibility, initially by using sire progeny tests for liver injury score, and later by directly using performance test GGT results (Morris *et al*., 1995a). The resistant and susceptible selection lines were retained until 2009.

Early genetic studies of host resistance to FE at the molecular level began over 20 years ago. One early protein candidate was transferrin (Morris *et al*., 1988). This protein appears to have an antioxidant role through its iron cofactor, and sires with different variants showed different degrees of FE resistance in

their progeny groups. Subsequently, a two-dimensional electrophoretic study of liver proteins in selection-line sheep was conducted (Lu *et al*., 1994), and different spots were found to associate with the resistant and susceptible lines.

One mechanism of FE cytotoxicity is believed to be the production of reactive oxygen species during redox cycling of the disulfide group in sporidesmin (Munday, 1989). These free radical species, which include hydrogen peroxide, superoxide and hydroxyl radicals, are harmful in biological systems (Halliwell and Gutteridge, 1990). Cellular antioxidant enzymes can protect against these radicals: for instance, superoxide dismutase converts the superoxide radical into hydrogen peroxide, which is reduced by catalase and glutathione peroxidase to water and molecular oxygen, and the glutathione cofactor required for glutathione peroxidase activity is maintained in its reduced form by glutathione reductase (Yu, 1994). Taking the candidate gene approach, microsatellite markers close to the genes for CuZn-superoxide dismutase, Mn-superoxide dismutase, glutathione peroxidase, glutathione reductase and ceruloplasmin were analysed for co-segregation with disease phenotypes in four FE outcross families described below. None showed any significant association with the FE trait (Phua *et al*., 1998). More in-depth and systematic studies were done on the catalase gene, which was mapped by linkage to ovine chromosome 15 (Phua *et al*., 1999). Although catalase did not fall within the quantitative trait loci (QTL) identified below, two nearby microsatellite markers (*OarSHP3* and *OarSHP4*) and an exonic single-nucleotide polymorphism (SNP) marker (*KP1*) showed significant differences in allele frequencies between the FE-resistant and FE-susceptible selection lines using a 'Peddrift' test (Dodds and McEwan, 1997). However, comparison of coding sequences of catalase complementary deoxyribonucleic acids (cDNAs) from resistant- and susceptible-line sheep showed only two silent mutations. Interestingly it was found that the activity levels of catalase and glutathione peroxidase in blood were significantly higher in FE resistant-line sheep than in susceptible-line sheep, and in contrast the reverse was seen for superoxide dismutase, whose blood activity level was significantly lower in the resistant than in the susceptible line (Hohenboken *et al*., 2004).

It is not known how zinc prophylaxis works to protect animals from sporidesmin toxicity. It appears that the protective mechanism does not require *de novo* gene transcription, as shown by cell culture experiment (Duncan *et al*., 2005). It is possible that zinc complexes with sporidesmin (Woodcock *et al*., 2001a,b) to abolish reactivity of the toxin with cellular macromolecules or to prevent generation of superoxide radical through redox cycling of the disulphide moiety. Alternatively, zinc may act through its biological roles providing structural components and cofactors of metallo-enzymes (Kambe *et al*., 2004).

In model studies with the yeast *Saccharomyces cerevisiae*, Bissinger and Kuchler (1994) reported that expression levels of *STS1* (also known as *pleiotropic drug resistance protein 5* (*PDR5*)) were associated with sensitivity to sporidesmin. The yeast *STS1* belongs to a superfamily of ATP-binding cassette (ABC) transporter genes, of which there are 48 gene members in humans, grouped into eight subfamilies (Dean *et al*., 2001). Specifically, *STS1* belongs to the mammalian ABCG subfamily (Sheps *et al*., 2004; Duncan *et al*., 2007), while the *ABCG1*, *4*, *5* and *8* genes are involved in lipid and cholesterol

homeostasis (Schmitz *et al*., 2001), and *ABCG2* is known to function as a xenobiotic transporter in humans (Staud and Pavek, 2005) and in cattle (Jonker *et al*., 2005). SNP markers were identified within ovine ABCG2 and were used to map the gene to ovine chromosome 6, to a region that contains some evidence of an FE QTL (Duncan *et al*., 2007). No non-synonymous SNPs were detected, indicating no differences in primary protein structure of the *ABCG2* gene between the resistant- and susceptible-line sheep. Although significant differences in allele frequencies of an intronic SNP marker were found between selection lines, no differential expression of the gene was observed in livers from resistant versus susceptible animals.

A more comprehensive approach to identify genes involved in FE resistance or susceptibility was a genome-wide experiment to screen for QTL in Romney sheep (Phua *et al*., 2009). Four first-cross sires were obtained by reciprocal matings of FE-resistant (R) and FE-susceptible (S) selection-line animals. The R × S sires were outcrossed to unselected Romney ewes to generate four half-sib families, with 120–170 progeny per sire. All progeny were dosed orally with a fixed dose rate of sporidesmin (at 0.13 mg/kg live weight), and their phenotypic responses were measured in terms of activities of GGT and glutamate dehydrogenase (GDH) in the blood. About 250 microsatellite markers, evenly distributed throughout the 26 sheep autosomes, were analysed in the four families for linkage to the GGT and GDH traits. This experiment detected one significant QTL on sheep chromosome 3 and four suggestive QTL on chromosomes 1, 8, 13 and 15 (Phua *et al*., 2009).

A complementary approach using microarray technology was also undertaken to examine gene expression profiles in the livers of resistant- and susceptible-line sheep. In the first microarray experiment, total liver ribonucleic acid (RNA) levels from sporidesmin-dosed resistant and susceptible animals were analysed using a 1500 bovine cDNA-array (Duncan *et al*., 2002). About 24 genes were found to be differentially expressed in livers of resistant versus susceptible sheep. In the second experiment, differential profiling of liver transcripts between four resistant and four susceptible animals, both with and without sporidesmin challenge, was performed using a 12K ovine cDNA-array (unpublished data). In total, 200–400 genes were differentially expressed. These genes covered a wide range of functions, with some encoding secreted proteins. In addition, some of them fell within QTL regions identified in the genome-screen experiments. However, attempts to confirm the differential expressions of 12 genes using an independent quantitative RT-PCR method showed only 4 to be significant.

A sporidesmin testing service called 'Ramguard' has been established for the New Zealand sheep industry, whereby candidate rams are performance-tested using oral dosing with the toxin under controlled conditions and veterinary supervision. The response is measured 21 days later, by analysis of a blood sample for GGT enzyme activity. The test has been offered annually for use by ram breeders since 1984 (Morris *et al*., 1994). In view of animal welfare considerations, a diagnostic DNA test would, however, be preferable to Ramguard in the long term, as it would minimize the invasiveness encountered with the performance test.

In dairy cattle, following earlier demonstrations in sheep that FE resistance was heritable, similar results have been reported (the latest heritability estimate for log GGT being 0.40 ± 0.04: Cullen *et al*., 2006). Genetic studies of FE resistance began in dairy cattle in 1989, by progeny-testing 65 bulls through their 2-year-old daughters (n = 1088) in herds where there was accidental exposure to FE. More recently, bulls have been progeny-tested by experimentally dosing young sons with sporidesmin (n = 572 sons, in 2002–2004), but since 2004, researchers have reverted to the cheaper method, by phenotyping daughters of known pedigree following natural FE challenge in commercial dairy herds (n = ~12,900 cows and young stock in 61 herds, in the autumns of 2004–2009). Many Friesian and Jersey sires have been ranked by these methods, and 228 sires now have an estimated breeding value, with a reliability of at least 0.60. Using DNA from sires, cows and bull calves that were extreme for resistance or susceptibility to FE, a marker study has been carried out with microsatellites at AgResearch Ruakura. A limited number of promising chromosomal regions are under assessment, and these are now being followed up by further genotyping. The ultimate test, which could be added to existing DNA testing of artificial-insemination bulls, will involve developing a panel of SNP results incorporated into a selection index for bulls.

In summary, FE resistance is a metabolic disease with a moderate heritability in sheep and dairy cattle. Industry initiatives to breed for increased tolerance are under way through challenge tests in ram-breeding flocks, but these initiatives will be assisted greatly when a diagnostic DNA test is available. In dairy cattle, developing an effective SNP test is the main priority.

Ryegrass Staggers

Predominantly a summer/autumn metabolic disorder, common in New Zealand sheep and cattle (Fletcher *et al*., 1999), RGS is caused by ingestion of the lolitrem-B toxin from perennial ryegrass (*Lolium perenne* L.) infected with the endophyte *Neotyphodium lolii*. The disease also occurs in southern Australia (Reed *et al*., 2005) and the USA (Tor-Agbidye *et al*., 2001). It causes neuromuscular incoordination in susceptible animals under stress, e.g. when mustered by sheep dogs, and when moving dairy cows for milking. It is of welfare concern and it is costly to farmers because it severely compromises grazing management. It has been estimated to cause financial losses exceeding NZ$88 million per year to sheep farmers in RGS-prone districts of New Zealand (C.A. Morris, 2004, unpublished data).

An RGS scoring system has been developed to combine data on severity and time of clinical incidence, and the latest heritability estimate for this score in sheep is 0.36 ± 0.04 (Morris *et al*., 2007). Susceptibility to the disease is also thought to be heritable in cattle (C.A. Morris, 1995, unpublished data). Divergent lines of sheep have been selected successfully for resistance or susceptibility to RGS since 1993. In the 2005 and 2006 lamb crops, where the two selection lines were grazing together, 2% and 6.5% of resistant-line lambs, respectively, showed signs of clinical staggers, compared with 94% and 91% of

susceptible-line lambs ($P < 0.001$). Using three first-cross sires, large numbers of backcrosses and outcrosses have also been generated from these genetic lines, phenotyped for RGS resistance and tissue samples have been stored for future molecular studies. Some of the detoxification pathways by which sheep can cope with RGS may be in common with those for FE, because there is a moderate positive genetic correlation (0.31) between resistance to the two diseases (Morris *et al*., 1995b). Additionally, there may be differences in resistance/susceptibility to ergovaline in these lines, as discussed below. A major advance in understanding the cause of RGS has been made recently by Imlach *et al*. (2008), at least in mice as a model, where it has been demonstrated that the large conductance calcium-activated potassium ion channel is the 'molecular target' for the lolitrem-B neurotoxin and a structurally related compound, paxilline.

Heat Stress

Tall fescue (*Festuca arundinacea*) infected with the endophyte *Acremonium coenophilum* produces ergovaline and other toxins that cause heat stress, along with reductions in prolactin concentration, feed intake, growth rate, milk production and fertility in susceptible animals (Schmidt and Osborn, 1993). The resulting disease, 'tall fescue toxicosis', is a serious problem in North America and in parts of Australia (see below). Breed differences in the depression of weight gains have been reported (Morrison *et al*., 1988) in Arkansas, and genetic differences in resistance (lower rectal temperature in response to a heat challenge) have been identified among sire groups of cattle in Missouri (Lipsey *et al*., 1992).

In model genetic studies with mice, divergent selection lines were created that were resistant or susceptible to the impact on post-weaning growth of a diet containing endophyte-infected fescue seed (Hohenboken and Blodgett, 1997). Growth, lifetime reproduction and mature size of resistant-line mice were less seriously compromised by the toxin-containing diet than were those of susceptible-line mice (Wagner *et al*., 2000). In a follow-up experiment with these lines, resistance to toxins in endophyte-infected tall fescue was also found to be associated with greater resistance to the FE toxin, sporidesmin (Hohenboken *et al*., 2000). The sporidesmin dose required to kill resistant-line mice was, on average, 35% greater than that required to kill susceptible-line mice. These data suggest a favourable genetic correlation between resistance to tall fescue toxicosis and FE, at least in mice, although these two toxins act on different target sites.

It has been hypothesized that some fungal toxins may work in tandem, so that their combined effect is greater than the sum of their individual effects. Reed *et al*. (2005) have suggested that ergovaline and lolitrem-B may be two such toxins. These authors reported severe effects in southern Australian states in 2002 where these two toxins coexisted. There were *c*.29,100 sheep deaths and *c*.450 cattle deaths on 224 farms thought to result from these toxins. High positive correlations have been reported in the USA between ergovaline and lolitrem-B concentrations measured in *c*.450 endophyte-infected perennial

ryegrass samples (Hovermale and Craig, 2001). Animals from the AgResearch RGS-selection lines described above were exposed to a heat stress challenge, following an artificial challenge with ryegrass seed containing 30 ppm ergovaline, and it was found that the resistant-line animals had rectal temperatures that were, on average, lower by 0.53°C than susceptible-line animals ($P < 0.05$) (M. Scannell, 2003, unpublished Honours project, Lincoln University, New Zealand). Thus selection for RGS resistance appears to have resulted in at least partial resistance to both ergovaline and lolitrem-B.

Extensive microarray studies at the University of Columbia-Missouri on rats (Settivari *et al*., 2006) and mice (Bhusari *et al*., 2006) have identified biochemical pathways thought to be associated with animal responses to toxins from the tall fescue endophyte.

Zearalenone

Zearalenone is a naturally occurring mycotoxin from the *Fusarium* species that grows on moist, dead plant material in many New Zealand pastures in autumn. In survey work, Garthwaite *et al*. (1994) found zearalenone at toxic levels in autumn on at least some pastures sampled over different districts throughout New Zealand. From more than 6000 samples tested 9% contained high enough zearalenone levels to depress ewe fertility, and another 35% were from 'at-risk' paddocks. Although it is a mycotoxin, the chemical structures of zearalenone and its metabolic breakdown products are similar to the reproductive steroid hormones. This enables the former to bind to the oestrogen receptors of mammals (Coulombe, 1993), interfering with the signal transduction and control functions of endogenous oestrogens. In adult sheep, because the mycotoxin is present at around mating time in autumn, the primary effect of zearalenone is to reduce ovulation rate and pregnancy percentage, leading to decreased lamb production (Smith and Morris, 2006). In contrast, in cattle, where the mating season in most New Zealand herds is not in autumn, the fertility rates of cows are apparently unaffected by zearalenone (Towers, 1996).

Zearalenone is metabolized in the liver and the breakdown products are excreted via urine and faeces. As its name implies, zearalenone is found commonly on maize, as well as pasture, and it may be a toxin of relevance to cattle and other livestock feeding on concentrate diets.

Repeatability and heritability of zearalenone

Three small trials have provided the opportunity to monitor urinary response, at different times after dosing with zearalenone, or following different dosing protocols. Results have been variable, giving correlations within animals of 0.82 (repeated sampling within a trial), and 0.27 and 0.23 for the same animals each monitored in two trials (C.A. Morris, 2005, unpublished data). Additional factors explaining this variability have yet to be identified. The partitioning of zearalenone breakdown products between urine and faeces has not been evaluated

genetically, but the concentrations of zearalenone breakdown products in urine have been shown to be heritable. A field test at AgResearch Ruakura measuring response to a pasture challenge, or to oral dosing with physiological levels of zearalenone, showed a heritability of 0.32 ± 0.10 in lambs and yearlings (Morris *et al*., 2005). In follow-up work in eight industry ram-breeding flocks, a challenge test was carried out in a similar way to the Ramguard sporidesmin testing service for FE (Morris *et al*., 1994). The heritability estimate for response to zearalenone under field conditions was 0.19 ± 0.07 (Amyes and Morris, 2008), using the toxin in seasons where there was expected to be minimal natural challenge. In that study, there were no significant genetic correlations between zearalenone response and the production traits measured (yearling live weight, yearling fleece weight and litter size at birth).

Pasture Bloat

Pasture bloat is a disease that mainly affects cattle, and is most commonly found when they graze on diets with high levels of clover, as found in New Zealand (Carruthers *et al*., 1987), western Canada (Howarth *et al*., 1991) and South America, especially Argentina (Basigalup *et al*., 2006). In susceptible animals experiencing bloat, the metabolic problem is an inability to eructate gases from the reticulo-rumen as quickly as they are produced. Bloat is thought to occur most often under conditions where there is fast release of breakdown products from clover leaves.

At AgResearch Ruakura two lines of Friesian × Jersey cross cattle were bred in order to study resistance/susceptibility to pasture bloat. The lines were selected divergently for increased or decreased susceptibility, for 30 years from 1972/73. Estimates of repeatability and heritability for single-record bloat score were 0.44 ± 0.02 and 0.19 ± 0.04, respectively, and the divergence achieved between lines was equivalent to 1.20 phenotypic standard deviations (Morris *et al*., 1997). The lines have been used to generate first-cross sires, and then backcrosses (1996–2002 calf crops) from dams of both selection lines. The backcrosses were phenotyped for bloat susceptibility, with DNA collected and stored for future use. Discovery of an apparent dominance effect for the bloat phenotype in the first cohort of backcrosses, such that the allele(s) for high-bloat susceptibility appeared to be recessive to those for low-bloat susceptibility, led to all subsequent backcrosses being created from matings to susceptible-line cows. To supplement animals/DNA samples from this research herd, a second DNA resource for studying bloat genetics in commercial dairy herds has been collected by AgResearch: in the spring seasons of 2001–2003, a total of 795 samples for DNA were collected from cows, all of which were said to have died of bloat. This resource, along with DNA samples from control animals, should yield an independent test of any association found between DNA markers and the bloat phenotype in the research herd.

A possible mechanism for the bloat–susceptibility differences between lines has been suggested, through the study of salivary proteins from high and low selection-line cattle (Rajan *et al*., 1996). A bovine salivary protein, now known

as bSP30, was found to have different abundance in the two selection lines. Two cDNAs (bSP30a and bSP30b) have now been cloned and sequenced, coding for alternate forms of a prominent protein in saliva. These proteins are thought to have originated as part of the parotid secretory protein/lipopolysaccharide-binding protein superfamily, also found in humans on HSA20q11.2 (Wheeler *et al*., 2002). Further study of the genetic control of the expression of bSP30 genes in cattle could provide insights into host differences in bloat susceptibility.

Minerals and Metabolites

Mineral deficiency

Although genetic variation in the concentrations of a mineral, a trace element or a metabolite is not an indicator of genetic variation in the occurrence of a specific disease per se, it could be a useful indicator of animals near an upper or lower threshold associated with clinical disease. For example, in New Zealand, Morris *et al*. (1990) found a heritability of 0.15 ± 0.06 for serum Mg concentration in dairy cattle (1454 lactating 2-year-old Jersey cows that were daughters of 65 sires), although it is not known whether the genetic outliers for low Mg were more prone to hypomagnesaemia. Heritabilities for serum K and Na in that study were low and not significantly different from zero. In the UK, Rowlands (1974) obtained heritability estimates of 0.93 ± 0.36, 0.74 ± 0.32, 0.40 ± 0.23, 0.28 ± 0.19, 0.26 ± 0.18 and 0.09 ± 0.12, for blood concentrations of haemoglobin, glucose, K, albumin, inorganic PO_4 and Na, respectively (231 Hereford × Friesian calves: 12 sire groups). In Texas, Greene *et al*. (1986) demonstrated significant breed differences in sensitivity to hypomagnesaemia among cows, and the authors were able to partition the breed differences into those caused by: (i) different Mg intake; (ii) different apparent Mg absorption rates; and (iii) different daily Mg requirements (which themselves were affected by physiological status). In Florida, Odenya *et al*. (1992) estimated whole-body amounts of serum Ca, P and Mg, measured in beef calves at weaning, and found moderate heritabilities of 0.39, 0.40 and 0.36, respectively. In New Zealand, Morris *et al*. (2006) reported heritability estimates for blood concentrations in beef cattle of 0.28 ± 0.08 to 0.35 ± 0.11 for copper, and 0.26 ± 0.13 for zinc. In Norway, Tveit *et al*. (1991) obtained a heritability of 0.11 ± 0.09 for the nadir Ca concentration in plasma in first-parturition dairy cows (18–30 h postpartum), and also a large negative genetic correlation (–0.49) between this value and milk yield (measured over weeks 2–5 postpartum). About three-quarters of the genetic variation in nadir Ca concentration remained unexplained by milk yield in that scenario. Heritability estimates for milk fever incidence (second and later parities) were also reviewed from the literature by Tveit *et al*. (1991). Estimates ranged from 0.07 to 0.42, and values increased with parity. In Norway, Heringstad *et al*. (2005) estimated heritabilities for milk fever incidence of 0.09, 0.11 and 0.13 for cows in their first, second and third lactations, respectively (372,000 Norwegian Red-sired cows).

In the UK, Pryce *et al*. (1997) estimated a heritability of 0.08 ± 0.01 for the incidence of milk fever, and an unfavourable genetic correlation of 0.19 ± 0.06 with lactation milk yield (15,280 records, comprising approximately 24% heifers).

In sheep, significant differences among five dam breeds and four lamb-sire breeds were observed (Wiener and Field, 1971) for blood concentrations of calcium, copper, phosphorus, magnesium and potassium, but not for sodium. Within-breed genetic variation was estimated, and offspring-dam heritability estimates ranged from 0.13 to 0.41 for the first five minerals above (average 0.21), although only one estimate was significantly different from zero (copper, with magnesium close to significance). Neary *et al*. (1998) reported a susceptibility locus for copper deficiency in sheep, close to the *Hb*-type locus. Campbell *et al*. (2003) reported significant QTL for bone density (on chromosomes 1p and 24), from computed tomography measures in Coopworth sheep, with heritabilities for long and flat bone densities being 0.64 ± 0.14 and 0.49 ± 0.14, respectively.

Ketosis

In the Norwegian study above, Heringstad *et al*. (2005) also estimated heritabilities for the incidence of ketosis in dairy cows, providing values of 0.14, 0.16 and 0.15 in the first three lactations, respectively. As with other disease traits currently included in the Norwegian dairy cattle recording scheme, it is expected that incorporating ketosis records into the selection index should lead to reductions in ketosis over time, despite a moderately low heritability, because the numbers of daughter records per sire are large. In practice, Heringstad *et al*. (2005) reported significant genetic improvement in ketosis incidence via sire selection over 20 years from 1976 onwards; relative weights of ketosis in the total merit index in Norway have ranged from 1.8% to 8.8% over this period. In the USA, Zwald *et al*. (2004a) studied farmers' records of ketosis incidence, and estimated heritabilities of 0.11 for ketosis in first lactation and 0.06 over all lactations (52,900 dairy cows; mean incidence, 10% per lactation). Correlations among sire breeding values (transmitting abilities) for ketosis and milk yield, fat percentage and protein percentage were all positive (unfavourable), although weak (0.12, 0.06 and 0.09, respectively) (Zwald *et al*., 2004b).

Foot Problems in Cattle

Zwald *et al*. (2004a) also estimated the heritability of lameness using data from the same cows described above, and obtained estimates of 0.07 for cows in first lactation, and 0.06 for all cow ages. Three other estimates reviewed by these authors were in the range 0.15–0.22. The UK study described above (Pryce *et al*., 1997) estimated a heritability of 0.026 ± 0.008 for lameness and an unfavourable genetic correlation of 0.29 ± 0.11 with lactation milk yield.

Perhaps as expected for a trait that might mean different things to different herdsmen or in different countries, the heritability estimates themselves seem to be of variable size.

Eye Defects in Cattle

In *B. taurus* cattle, infectious bovine keratoconjunctivitis (IBK or 'pinkeye'), an infectious disease and ocular squamous carcinoma (or 'cancer eye') are important eye defects. As IBK is not discussed elsewhere in this volume, we note (from Snowder *et al*., 2005) that: (i) there are breed differences in IBK incidence; (ii) it is a heritable trait, with heritability estimates in pre-weaned beef calves averaging 0.12; and (iii) subsequent studies in the same laboratory (Casas and Snowder, 2008) have established the presence of a QTL for pathogenic disease (including IBK) on cattle chromosome 20.

Heritability estimates for eyelid pigmentation have been reported by French (1959), with values of 0.64–0.89. These are important because increased eyelid pigmentation is associated with reduced levels of cancer eye or precursor lesions (French, 1959; Vogt *et al*., 1963). The Hereford appears to be highly sensitive to cancer eye (French, 1959; Anderson, 1963), and heritability estimates for cancer eye (reviewed by Morris, 2007) are: Blackwell *et al*. (1956) 0.17 and 0.29, Vogt and Anderson (1964) an average of 0.14, Russell *et al*. (1976) 0.10 ± 0.08 (incidence data) and 0.30 ± 0.09 (number of tumours), giving an average across studies of 0.16 for cancer eye incidence.

Discussion

Seven groups of traits in cattle have been reviewed in this article (FE, bloat, mineral deficiencies, milk fever, ketosis, foot problems and eye defects) and four in sheep (FE, RGS, zearalenone infertility and mineral deficiencies). Averaging the heritability estimates for each trait, and then calculating an unweighted average across the disease categories, gave values of 0.21 in cattle and 0.30 in sheep. These estimates are high enough to suggest that most traits could be changed by genetic selection if there was sufficient economic incentive.

Any artificial selection to be applied to a disease trait requires the ranking of animals for that trait, or the use of an indicator. It is at this point that the herd- or flock-owner often shows reluctance to score animals from the breeding herd/flock, whether using natural or artificial challenge, because the challenge may affect the future target performance levels achievable with those breeding stock. For this very reason, and for animal welfare, it is desirable to identify DNA markers for any important disease trait, and then to offer breeders the opportunity to apply marker-assisted selection to the trait. Nevertheless it will still be necessary to provide an artificial challenge to some animals and to rank them, so that a gene discovery phase can be carried out in the first place, followed by a validation phase for the markers identified.

Conclusions

This review has concentrated mainly on within-breed genetic differences in resistance or susceptibility to metabolic diseases, although the original observations highlighting the involvement of host genetic differences were often from anecdotal reports of breed differences. Almost all metabolic diseases studied in cattle and sheep had a heritable component, with heritabilities ranging from low to medium in magnitude. Most of the traits could be improved through genetic selection, if the testing and selection costs could be justified by extra net returns.

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16 Genetics of Metabolic Diseases in Poultry

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Summary

The importance of non-infectious disorders in poultry is reviewed and evidence for genetic variation is summarized. Many production and welfare-related problems have a significant environmental component, and care is necessary in adducing a solely genetic cause. Nevertheless, substantial genetic variation for many of these traits exists, and poultry breeding companies have applied selection pressure to reduce the propensity for musculoskeletal disease in parallel with improvements in the management of growing birds. Problems of trait measurement for damaging pecking and feed restriction in breeding birds remain to be solved, but it is suggested that recent developments in statistical methodology and also in whole-genome selection using dense deoxyribonucleic acid (DNA) markers will provide breeders with effective and powerful tools to reduce their importance in the future.

Introduction

Broiler and layer chickens, turkeys and ducks have been the subject of intense genetic selection for over 60 years, and this selection has resulted in remarkable changes in productive efficiency. Alongside these changes, the frequencies of idiopathic diseases have increased and courted controversy from welfare groups in addition to their adverse effects on productivity. Considerable research efforts have been made by scientists and animal breeders to identify the underlying causes and to provide solutions to the problems that they represent. These structural and metabolic diseases have been reviewed in depth in several publications, and the purpose of this chapter is to summarize the recent literature on the genetic basis of disorders in modern poultry.

 Breeding for Disease Resistance in Farm Animals, 3rd Edition (eds S.C. Bishop *et al.*)

Skeletal Disorders in Broilers and Turkeys

Background

The aetiology of many musculoskeletal disorders is complex and usually involves an interaction between a genetic predisposition to the disorder and the environment in which birds are kept. For example, virtually all non-infectious skeletal diseases affecting broiler chickens are absent in broiler breeders that are feed-restricted (see below). Some skeletal diseases are easily induced by specific nutrient deficiencies or imbalances, particularly of calcium, phosphorus and vitamin D, and still others are associated with infectious organisms. Care is therefore required in adducing a genetic basis for a particular condition. There are several excellent reviews of skeletal disease in poultry (Duff, 1990; Bradshaw *et al*., 2002; Whitehead *et al*., 2003), and a good background may still be found in the book edited by Whitehead (1991). A comprehensive review of muscle abnormalities in poultry is given by Mahon (1999).

Poultry breeders have placed increased selection effort into reducing leg disorders in the last 20 years through culling of birds that had less than perfect locomotion and leg bone structure. Bentley (2006), for example, showed that 50% of the potential selection pressure for weight gain was lost through selection for fitness in a pedigree flock of turkey breeders. Poultry producers have also addressed nutrient deficiencies and management practices, particularly in providing broiler chickens and turkeys with a longer dark period and by limiting early growth rate in broilers, to minimize the incidence of leg weakness (Classen, 1992).

Walking ability and welfare

Welfare concerns about broiler leg health prompted the development of a subjective scoring system for assessing the walking ability of broiler chickens (Kestin *et al*., 1992). A score of 0 (no problem) to 5 (unable to walk) was used to assess walking ability or gait of commercial broilers as an index of welfare and the potential for pain from musculoskeletal disorders. However, little attempt has been made to relate these subjective scores to underlying pathology (e.g. developmental or infective) and they remain controversial. Garner *et al*. (2002), for example, found no relationship between gait score and tibial dyschondroplasia (TD).

The welfare of birds that move with difficulty or are unable to walk is clearly compromised since, besides the potential painfulness of the condition, they are unable to feed or drink properly. However, birds with moderate gait scores (2 or 3) reflect normal changes in gait related to conformation (Corr *et al*., 2003a,b) and are not necessarily indicative of pain or poor welfare. Evidence that birds with moderate gait scores experience pain during locomotion (McGeown *et al*., 1999; Danbury *et al*., 2000) is not definitive. The heritability of the traditional gait score in commercial broiler flocks is close to zero (Dr M. Cooper, March 2010, personal communication), whereas the heritabilities of skeletal conditions that contribute to gait score are moderately

high (see below). Nevertheless, large differences in average gait scores between commercial broiler crosses have been observed (Kestin *et al*., 1999).

Varus/valgus deformity (VVD)

Varus (hocks out, feet in) and valgus (hocks in, feet out) deformities are commonly associated with compromised walking ability. Le Bihan-Duval *et al*. (1996) determined heritabilities of 0.16 and 0.29, respectively, for susceptibility to valgus deformity in two lines of broilers when estimated from the sire and maternal-grandsire components, and 0.40 and 0.35 when estimated from the dam component of variance. For varus deformity, estimates of heritability were 0.21 and 0.24 using the sire and maternal-grandsire variance component and 0.30 and 0.26 from the dam component. The average estimated genetic correlations between valgus and varus obtained from the male and female components were small to moderate (–0.31 and 0.07). These results suggest that each deformity has a different cause and that pooling them is unlikely to improve the accuracy of broiler evaluation, consistent with the low heritability of gait score noted above. However, Akbas *et al*. (2009) recently estimated the heritability of VVD to be 0.72. Le Bihan-Duval *et al*. (1997) reported very low genetic correlations of 0.05 and 0.01 between body weight at 6 weeks and susceptibilities to valgus and varus disorders. Therefore, inclusion of leg defects in breeding schemes would not adversely affect selection for increased production traits. The small positive genetic correlation between varus and breast conformation suggests that selection for a high proportion of breast muscle may partly have caused an increase of this disorder in the past.

Rotated tibiae and crooked toes

Rotational deformation of the long bones is observed when the natural growth of the tibia or femur is exaggerated, causing abnormal limb and joint angles. Selection experiments in the 1970s and 1980s to reduce the incidence of twisted legs were successful, suggesting a moderate heritability (Sørensen, 1992). Sørensen (1992) also quoted similar experiments to reduce crooked toes in White Leghorns and broiler chickens. There appear to be no recent estimates of genetic parameters for these disorders.

Tibial dyschondroplasia (TD)

Dyschondroplasia is an abnormality of the growth plate of the long bones and typically occurs in the proximal end of the tibia in rapidly growing poultry. The lesion appears grossly as a plug of cartilage that has not ossified and may be associated with abnormal bone growth. Histologically the lesion is characterized by the accumulation of transitional chondrocytes that form a mass of avascular cartilage beneath the layer of proliferative chondrocytes. Lesions develop at about 2–3 weeks in broilers and at 9–12 weeks in turkeys.

The frequency of TD responds rapidly to genetic selection for higher or lower prevalence and is readily induced by manipulation of the dietary concentrations of calcium and phosphorus and by deficiencies of vitamin D. Bartels *et al*. (1989) used a portable X-ray machine (Lixiscope) to measure TD in live birds and proposed its use as a selection tool in pedigree flocks. Ducro and Sørensen (1992) used the Lixiscope in a divergent section experiment and reported a heritability of 0.33 at 4 weeks of age. In a similar experiment over seven generations, Wongvalle *et al*. (1993) reported a realized heritability of TD in the high line at 7 weeks of age of 0.44, but there was no effect of genetic selection in the low line. Zhang *et al*. (1995) reported negative genetic correlations between TD and body weight of −0.65 and −0.46, respectively, estimated from sire and dam components of variance in a seven-generation selection experiment. Their results suggested that maternal effects on the incidence of TD were significant, possibly as a result of differences in egg size or nutrient deposition in the albumin and yolk. In contrast to these results, Kuhlers and McDaniel (1996) estimated the heritability of TD at 7 weeks of age to be 0.42 and a genetic correlation with body weight near zero, also in a seven-generation experiment. Akbas *et al*. (2009) recently reported a heritability of 0.21 in Hubbard broilers. Commercial breeding companies have selected successfully against TD using the Lixiscope, and the prevalence in commercial broilers has declined over the last decade.

Destructive cartilage loss (DCL)

Thickened epiphyseal growth plates with avascular cartilage and ischaemic degenerative changes (necrosis, clefts, haemorrhage and proliferative changes) are frequently observed in broiler and turkey breeders. Is it associated with lameness through cartilage loss and avulsion of intra-articular ligaments. It may also be associated with femoral head separation and lameness in broilers, and predispose affected birds to infective arthritis.

The cartilage of the hip joint in adult broiler male-line males and in both male and female turkeys of male lines is susceptible to DCL, and the prevalence increases with age (Duff and Hocking, 1986). The antitrochanter (a dorsocaudal extension of the acetabulum that articulates with the femoral trochanter) is particularly vulnerable to idiopathic disease. Histopathology shows retained avascular, cellular cartilage with clefts that eventually lead to cartilage loss and exposure of the underlying bone.

Turkeys and male broiler breeders with DCL were given analgesia and the birds showed increased motivation and walking in early but not later experiments (Duncan *et al*., 1991; Hocking, 1994; Hocking *et al*., 1999). Lines vary in their susceptibility to antitrochanteric degeneration and the prevalence is higher in lines selected for high body weight (Hocking and Lynch, 1991; Hocking *et al*., 1996; see Table 16.1), but there are no recent reports of the prevalence of antitrochanteric degeneration in commercial turkeys or broilers. It is possible that improved general skeletal health has significantly reduced DCL in current flocks of breeding poultry, as the prevalence in male broiler breeders was low (10%) in an unpublished experiment conducted by the author in 1998 and cartilage lesions were relatively mild.

Table 16.1. Proportions of male and female turkeys with antitrochanteric degeneration of the hip joint at 54 weeks of age. Male and female turkeys of the large-sire line were fed *ad libitum* (AL) or restricted (R) to control body weight. (From Hocking *et al.*, 1998.)

Line	Gender	Feeding	Body weight (kg)	Proportion affected (%)
Sire line	Male	AL	26.7	81
		R	23.5	25
	Female	AL	14.9	88
		R	12.9	50
Traditional	Male	AL	11.5	0
	Female	AL	6.1	0

Spondylolisthesis

Spondylolisthesis is caused by a deformation of the T4 vertebra of the spinal column that leads to a pinching of the spinal cord and subsequent paralysis of the hind limbs. Affected birds are characterized by a sitting posture, resting on their hocks with the feet slightly raised. Early research revealed marked differences between genetic lines and the incidence was increased by genetic selection (Riddell and Howell, 1972; Riddell, 1973). The condition is uncommon in modern poultry probably as a result of selection against the condition in pedigree breeding.

Slipped tendon and ruptured tendons and ligaments

Slipped tendon occurs as a sequel to deformed bone growth, particularly of the tibia, that leads to displacement of the gastrocnemius tendon. Rupture of the gastrocnemius tendon above the hock is a common cause of lameness in rapidly growing broilers. Ruptured ligament in lame breeding males has also been reported (Duff and Hocking, 1986; Duff *et al.*, 1987).

Muscle Disorders in Broilers and Turkeys

Deep pectoral myopathy, focal and acute myopathy

Deep pectoral myopathy (Oregon or Green Muscle disease) is caused by an anatomical predisposition to ischaemia after physical exertion (wing flapping). It is caused by rapid expansion of the suprachoracoid muscle that is constrained by the muscle sheath, cutting off the blood supply and leading to muscle death. Additionally, there is evidence that the capillary supply to the breast muscles in turkeys is poor and may exacerbate the problem (Sosnicki *et al.*, 1991). It was noted as a commercial problem in the 1970s and 1980s, particularly in turkeys, and is no longer commonly seen, most probably because stress at handling and slaughter has been reduced.

Focal and acute myopathies are similar to deep pectoral myopathy and usually occur in young rapidly growing male broiler breeders in both red and white muscles (Mahon, 1999).

Pale soft exudative muscle

Pale soft exudative muscle has been described in broiler chickens and turkeys and results in poor water-holding capacity and pale, tough meat. It may partially be related to heat stress during transport prior to slaughter. Compared with layers, broiler chickens are more susceptible to heat stress at the same age or body weight (Sandercock *et al*., 2006). In the absence of heat stress, broiler and turkey muscles are characterized by pathological changes including focal fibre necrosis, hypercontraction and alterations in cell membrane integrity (Mahon, 1999). The genetic basis for these problems is related to selection for high growth rates and associated muscle mass but the physiological mechanisms are not understood. It is possible that these muscle pathologies are related to painful muscle conditions.

Osteoporosis in Laying Hens

Osteoporosis in laying hens is associated with loss of bone mineral during the laying period, resulting in poor bone strength and bone fractures. Broken bones occur during the laying period, at depopulation, during transport and during the slaughter process (Gregory and Wilkins, 1992). In a study comparing traditional breeds with commercial laying hens (Hocking *et al*., 2003), traditional breeds were characterized by lower rates of egg laying and by higher strength and radiodensity of the tibiae and humeri at the end of lay, whereas at 30 weeks of age no differences between traditional and commercial birds were observed. Data such as these strongly suggest that genetic selection for persistently high rates of egg laying, which requires a high degree of calcium mobilization from bones for egg shell formation over an extended period of time, has resulted in the very high rates of the disease observed in commercial laying hens.

The heritability of traits related to bone strength was moderately high, and Bishop *et al*. (2000) developed a selection index based on tibia strength (heritability = 0.45), humerus strength (0.30) and keel radiographic density (0.49) in a flock of White Leghorn chickens. The selection traits were measured at the end of the laying period and two lines were selected respectively for high and low values of the index. All bone characteristics used in the selection index responded rapidly to divergent selection. In the last (sixth) generation, the lines differed by 25% in tibial strength, 13% for humeral strength and 19% for keel radiographic density and the realized heritability of the index was 0.40. The overall prevalence of bone fractures was decreased in the high line, being reduced by a factor of 6 in the humerus. There was a correlation of 0.92 between tibia strength and the overall incidence of bone fracture. Taken together these results suggest that genetic selection to increase skeletal strength and decrease the propensity for bone fractures during and at the end of lay is

possible. However, methods to implement these findings that do not rely on extensive dissection of hens are required to accomplish this goal.

The lines developed by Bishop *et al*. (2000) were subsequently crossed and 372 F_2 chickens phenotyped for the bone index and component traits. Microsatellite DNA markers were used to genotype the flock, and one significant QTL on chromosome 1 was identified that explained 34% of the trait standard deviation for tibiotarsal breaking strength (Dunn *et al*., 2007). If the gene(s) and mutation(s) underlying this large QTL could be identified it would greatly facilitate marker-assisted selection to improve the welfare of laying hens by decreasing the propensity for bone fractures.

Integument

Foot pad dermatitis (FPD)

The prevalence of contact dermatitis increased after the ban on the use of animal products in poultry diets. The main alternative protein source – soybean meal – has high concentrations of potassium that increase water intake, which in turn results in poor (wet) litter. The most significant current welfare problem in turkeys and broiler chickens is FPD. It has long been known that wet litter increases FPD, but it has only recently been shown that high litter moisture per se is sufficient to cause lesions (Mayne *et al*., 2007a). The condition is an inflammatory response and the lesions do not harbour evidence of invasion by bacterial, viral or fungal organisms (Mayne *et al*., 2006, 2007b). Slower weight gain in affected birds has been taken to be indicative of pain (Martland, 1985; Mayne *et al*., 2007a). Management changes to reduce litter moisture are effective in decreasing the prevalence of FPD and differences between genotypes are reputed to exist. Akbas *et al*. (2009) reported a heritability of 0.34 for susceptibility to FPD, which is similar to that found by Kjaer *et al*. (2006), who reported a heritability of 0.31 and a low genetic correlation with body weight (–0.08). These results suggest that selection against susceptibility to FPD should be possible.

Hock and breast burn

Hock burn is probably similar to FPD but has not been investigated to the same extent. It is caused by exposure to wet litter and has a similar management solution. Breast lesions take two forms: focal ulcerative dermatitis (FUD or breast buttons) and sternal bursitis (SB or breast blisters). FUD follows abrasion of the breast skin, causing oedema and thickening of the skin over the breast. SB is caused by local skin infection, probably through a puncture wound to the skin. The prevalence of both conditions may be exacerbated by sharp litter which abrades or punctures the breast skin that happens to be in close contact with the litter. Kjaer *et al*. (2006) reported a heritability of 0.08 for hock burn and a low genetic correlation with body weight (0.17). Akbas *et al*. (2009) estimated a heritability of 0.17 for hock burns.

Cardiovascular Disease

Ascites

Ascites is the accumulation of ascitic fluid in the abdominal cavity and results in listlessness and a slow death or condemnation of broiler carcasses at the slaughter plant. It is the consequence of dilatation and hypertrophy of the right side of the heart, which leads to cardiac failure and changes in liver function (Julian, 2005). Ascites is associated with birds kept at high altitude or growing very rapidly at low temperatures. It is generally considered to be caused by insufficient oxygen supply or cardiovascular function, and it has a genetic component. Heritability estimates for susceptibility to ascites are low, in the range of 0.1–0.2 but higher in males (0.2–0.4) (Moghadam *et al*., 2001; Navarro *et al*., 2006a). There is evidence for the segregation of genes of large effect in broiler populations (Druyan *et al*., 2005; Navarro *et al*., 2006b; Druyan and Cahaner, 2007), and Druyan *et al*. (2007) selected lines for resistance and susceptibility to ascites. Troponin T is a cardiac-specific plasma metabolite that is an early indicator of cardiac disease (Maxwell *et al*., 1994). Maxwell *et al*. (1998) determined a heritability of 0.38 for plasma troponin T concentration at 12 h post-hatch from a parent–offspring regression, suggesting that it could be used as an early selection tool.

Quantitative trait loci (QTL) have been identified for traits related to ascites susceptibility in broiler chickens. Rabie *et al*. (2005) identified genome-wide significant QTL on chromosomes 2 (GGA2), 4 and 6 in an F_2 broiler cross. The most significant QTL were located on GGA2 at 325–421 cM. Navarro *et al*. (2005) reported the location of a QTL for an ascites-related trait (total blood cell count) on GGA2 but at a different location (67–273 cM). These authors also identified a QTL on GA11 for troponin T at 6 weeks of age in their F_2 broiler–layer cross. There is no prior reason to expect that QTL should be similar in different crosses and their confidence intervals are very large, making identification of the gene(s) involved a significant challenge. The use of these associations in marker-assisted selection is not feasible at the present time.

Genetic selection against susceptibility to ascites has been undertaken by broiler-breeding companies. Selection criteria have been based on oxygen saturation using an oximeter reading of the colour of blood coursing through a wing vein. Other methods have included selection at high altitude and under low environmental temperatures. The prevalence of ascites has been greatly reduced in commercial flocks through genetic selection and better environmental management, and ascites is no longer considered a significant risk factor in broiler flocks.

Sudden death syndrome

Moghadam *et al*. (2005) reported heritabilities of 0.25 and 0.30 for sudden death in two lines of broiler chickens and heritabilities of 0.30 and 0.40 if only males were used in the calculations. The genetic correlations with body weight in the two lines were 0.30 and 0.27.

Disorders of Breeding Poultry

Multiple ovulation

Broiler chickens, turkeys and ducks are relatively heavy at maturity and develop large ovaries in which more than a single ovum develops to ovulation. Multiple ovulation interferes with the successful capture of the yolks by the oviduct, leading to internal ovulation. If one or more yolks enter the oviduct, the formation of the eggshell is adversely affected, resulting in soft-shelled, membranous, defective or double-yolked eggs that cannot be incubated. The net result is that the production of hatching eggs is very poor (Table 16.2). Multiple ovulation in commercial broiler breeders and ducks is effectively controlled by limiting body weight gain, but this strategy is ineffective in turkeys (Hocking, 2009). Selection experiments have shown that there is a positive genetic correlation between growth and ovulation rate. The welfare of feed-restricted broiler breeders has been questioned, particularly as the required degree of feed restriction has increased with the selection for growth rate in broiler chickens (Renema *et al*., 2007). A genetic solution that reduces ovulation rate and permits an increase in body weight that optimizes welfare, without compromising productivity, is required to resolve this issue.

Sudden death

Overall mortality in broiler breeder hens fed *ad libitum* is high (Table 16.2). If the birds are reared in the same feed and photoperiod environment as conventional feed-restricted birds, then a large increase in mortality occurs mainly during the laying period and is associated with sudden death, apparently from cardiovascular failure. It is assumed that this is related to the heavy muscle mass of these birds because it is decreased by feed restriction after 37 weeks of age in birds fed *ad libitum* and does not occur in commercially feed-restricted birds. The latter statement must carry the caveat that sudden deaths occasionally occur in the period immediately after the onset of lay in feed-restricted broiler breeders and are generally related to insufficient dietary calcium or phosphorus, or an imbalance of these minerals.

Table 16.2. Final body weight, mortality, egg production, hatchability and feed intake during the rearing and laying periods of broiler breeder females fed *ad libitum* or feed restricted from hatch to 60 weeks of age. (From Hocking *et al*., 2002.)

Trait	*Ad libitum*	Restricted
Body weight (kg)	5.3	3.7
Mortality (%)	46	4
Egg production (*n*)	58	157
Feed intake (g/day)		
0–24 weeks	163	63
24–37 weeks	192	157
37–60 weeks	142	151

It is not known whether there is genetic variation for resistance to physiological stress in adult birds fed *ad libitum*. However, broiler, turkey and duck breeders are fed to maintain health and fitness either through feeding a low energy–low protein diet or by feed restriction, and it is not a common problem in commercial flocks.

Behavioural Disorders: Feather Pecking, Removal and Cannibalism in Laying Hens

Feather pecking is a general term that covers behaviours that involve hens manipulating the feathers of other hens, plucking out feathers or causing skin lesions, all of which may be associated with cannibalism, where hens eat the blood and tissue of other hens. Hens may also peck at and remove their own tissue from the damaged area, typically around the tail, vent and ventral abdomen but also including the feet and legs. It is not a new disorder but has gained importance as producers have changed from battery cages to free range and aviary systems, where the problem is ameliorated by partial beak amputation. There is evidence that selection practices such as trait measurement in single-bird cages and selection for early sexual maturity may increase the prevalence of the disorder.

There is substantial evidence for between-breed genetic differences in the propensity for the behaviour (Kjaer, 2000; Hocking *et al*., 2004), and lines of chickens have been developed by genetic selection for feather-pecking activity that show low or high levels of feather pecking in relation to an unselected control line (Kjaer *et al*., 2001). Heritabilities of feather pecks and feather-pecking bouts have been reported to be in the range of 0.10–0.17 (Su *et al*., 2005). In another study, group selection reduced the incidence of beak-inflicted injuries and mortality from an initially high level of 68% to 9%, by the third generation (Muir, 1996). The realized heritability was 0.65, suggesting that a major gene may have been involved in this population. Improved methods of selection by application of group selection theory combined with trait measurement in family groups may result in a reduction of this problem in the medium to long term (Muir, 2005; Bijma *et al*., 2007a,b).

Conclusions

Experimental data and analyses of commercial flock data suggest that significant genetic variation exists for many idiopathic disorders in poultry. Following is a summary of major disorders, their solutions and future challenges.

Genetic selection to decrease the prevalence of musculoskeletal disorders in growing broilers and turkeys, combined with improved management, has prevented further increases and may have resulted in a significant reduction in lameness. There are few public data from commercial flocks to substantiate these claims, and this is an unsatisfactory situation. Critical evaluation of the significance for broiler welfare of the modified gait of these birds is also required.

Management changes to ensure dry litter would greatly diminish the prevalence of contact dermatitis in growing birds, which is a major welfare issue in

commercial flocks. However, genetic selection to decrease the propensity for FPD is likely to be effective and may help to achieve the goal of relative freedom from these disorders in commercial situations.

Decreasing body weight of adult breeding turkeys and broiler chickens leads to a significant reduction in musculoskeletal disease (Hocking and Duff, 1989; Hocking, 1990; Table 16.1). Body weight control in commercial flocks has decreased the significance of these disorders in broiler and turkey breeders but, as for growing stock, there are no recent estimates of the prevalence of musculoskeletal disorders in commercial flocks.

The problem of substantial feed restriction in broiler breeders and selection to increase bone strength in laying hens requires the development of effective selection tools: whole-genome selection (selection based on trait–DNA marker associations determined in experimental populations, as proposed by Meuwissen *et al*., 2001) holds out great promise in this area. Group selection theory will undoubtedly be used in laying hens and may also be useful in decreasing feather pecking and cannibalism in other poultry.

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