

Foresight

Infectious Diseases: preparing for the future

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**S11: State-of-Science Review -
Host Genetics and Engineering: the genetics of
host responses to infectious diseases
in farmed animals**

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Introduction

All livestock production systems are influenced substantially by both genetic and environmental factors and their interaction. Disease imposes an important set of problems within these systems, including enhanced costs, production losses, uncertain food security, depleted income and diminished animal well-being. Some diseases, and the strategies used to manage them, directly impact on human health. Partial costing estimates of the impact of animal disease are 17% of turnover within the livestock sector of the developed world and 35–50% in the developing world.

There are various disease management options, including vaccination, chemotherapy, biosecurity, improved husbandry and genetic change (of the host). Each of these can partly manage disease problems, and the use of any one option has consequences for the production system as a whole. The specific advantages of incorporating genetic elements into the management of livestock diseases include low input and maintenance costs once the strategy is established, and permanence and consistency of effect. Together, these attributes result in genetic approaches that could, potentially, contribute to sustainable disease management. However, genetic approaches to disease management generally lend themselves more closely to disease **control** rather than **detection** or **identification**.

There are a number of important livestock diseases in livestock where genetic approaches have been shown to be effective in assisting in disease control in: developed and developing countries; intensive and extensive systems; and in subsistence and market-oriented enterprises. Successfully managed diseases have included tick infestations, helminth infections and Marek's disease. Additional diseases where genetics is currently being used as a component in management include trypanosomosis, mastitis, scrapie and *Escherichia coli* diarrhoea in pigs. However, the potential list of diseases for which host genetics may play a role is much wider than this. Host genetic variation in resistance has been demonstrated for more than 50 diseases, and, in almost every case where there has been a systematic investigation, genetic variation in host resistance or tolerance has been demonstrated.

Options for incorporating genetics into disease management strategies range from simple (e.g. choice of appropriate breed) to sophisticated (e.g. use of molecular markers). In the future, options may include the use of transgenic technologies. However, the choice of the appropriate technology depends on the nature of the disease, the infrastructure within which the particular livestock industry operates, and societal attitudes towards technology. In general, genetic strategies will be justified when (i) there is a disease of major importance, (ii) current control strategies are not adequate, sustainable or cost-effective, and (iii) available animals do not cope with these disease challenges. A successful genetic strategy should cost-effectively reduce the impact of the disease by altering its epidemiology in a reasonable time period.

Pressures due to many factors, including economics, legislation, the decreasing effectiveness of current intervention strategies, food-safety zoonotic concerns

and new diseases arising from climate change and increased human mobility, will force breeders to consider genetic solutions to a wider range of diseases. At the same time, through a combination of genome mapping and functional genomics, researchers will elucidate genes' underlying differences in resistance. These technologies, along with an understanding of the epidemiological impact of increasing resistance, should provide breeders with the tools they need to increase genetic resistance to a variety of diseases.

Scope of review

This review considers the following issues:

- the range of diseases for which genetic variation in host resistance (or tolerance of infection) is apparent within the three target regions, i.e. the UK, China and sub-Saharan Africa
- factors that determine whether or not the exploitation of host genetic variation in resistance (or tolerance) makes an appropriate contribution to disease management. Interaction with other disease control strategies and long-term sustainability are critical considerations
- options that may be used to exploit host genetic variation, and whether or not they are appropriate for specific diseases/livestock production systems/environments. These include:
 - conventional breeding techniques (choice of resistant breeds or individuals)
 - genetic markers or exploitation of identified 'resistance genes'
 - engineering techniques, such as transgenesis or RNAi, and the definition of circumstances when these approaches are feasible or appropriate
- technologies and steps that are required to distinguish resistant genotypes (by region)
- technologies and steps required to enable creation and/or exploitation of resistant genotypes
- risks arising from the use of host genetics in the control of infectious disease.

Focus on control

This review focuses more on the role of genetics in disease control than on the detection and identification of disease. This is because it is in the control of disease that host genetics has the more obvious role to play, and also where a much greater body of research has been undertaken, both in animals (the focus of this review) and plants. The use of host genetics in disease identification is discussed, although the conclusions drawn are necessarily more speculative. However, identification and control are not independent: it should be recognised that using animals with enhanced resistance may reduce the need for identification and diagnosis as disease prevalence should reduce as a consequence.

Infectious animal diseases in the target regions

Genetics of disease resistance

For the purpose of this review, we define infection as the colonisation of a host animal by a parasite, where 'parasite' is a general term to describe an organism with a dependence on a host. Parasites will include pathogens or microparasites with a direct dependence on the host, such as viruses, bacteria and protozoa, as well as macroparasites that complete some part of their lifecycle external to the host, such as helminths, flies or ticks. We define disease as the side-effects of infection by a parasite or pathogen. Disease may take several forms – acute, sub-acute, chronic and sub-clinical – which may or may not be debilitating.

In terms of disease resistance, it is also necessary to distinguish between resistance *per se* and tolerance. An individual host may be infected but may suffer little or no harm. This is known as tolerance. In contrast, resistance is the ability of the individual host to resist infection or to control the parasite lifecycle, e.g. by reducing the proliferation of the virus or bacteria within the host. The distinction between resistance and tolerance is important when considering the impact of breeding for disease resistance. In general terms, if genetic improvement is made in host resistance to infection, there will be a reduction in the transmission of infection to other animals. Conversely, genetic improvement of tolerance may reduce clinical signs of disease but may not reduce the transmission of infection to other animals or even to humans in the case of zoonotic infections. At worst, genetic improvement of tolerance may mask clinical signs of disease in infected animals and can make disease identification more difficult.

Evidence for genetic variation in disease resistance or tolerance

There is considerable evidence for genetic variation in disease resistance, i.e. the ability of the host animal to control the extent of infection or the ability of the host animal to tolerate infection. Of more than 50 livestock diseases (Table 1) for which there is documented or strong anecdotal evidence of

genetic variation in host resistance or tolerance (Bishop 2004; Gibson 2002), 19 appear in the lists of diseases notifiable to the OIE (Office International de Épizooties). The examples include all major livestock species, as well as all categories of parasite or pathogen. Although it is often not clear whether the observed genetic variation is for resistance to infection, tolerance of infection or a combination of both, we conclude that, for almost every disease for which appropriate studies have been performed, there is evidence for host genetic variation in either resistance or tolerance.

Breeding for disease resistance

Breeding for disease resistance should be considered as part of a wider disease control strategy. Such strategies may include interventions or decisions affecting the animal, the pathogen or the environment. For example, the animal may be influenced by means of vaccination, the selection of resistant animals or the culling of infected animals. Pathogen-based interventions include the use of chemotherapies such as antibiotics or anthelmintics. Environmental interventions include biosecurity, sanitation, improved husbandry, etc. But, despite the range of disease control options, there are many continuing challenges relating to animal health and disease. Many previously-used control strategies are now less available due to legislation (e.g. prophylactic use of antibiotics) or the evolution of the pathogen to avoid the control strategy (e.g. antibiotic or anthelmintic resistance). New issues arise continually, and some disease control problems simply remain unsolved.

We illustrate the potential for breeding for disease resistance by reference to a few diseases that are notifiable to the OIE. It should be recognised that conventional breeding techniques are limited in terms of the number of diseases they can potentially deal with at any one point in time, unless underlying mechanisms of the innate immune response are identified, which may allow resistance to several diseases to be enhanced simultaneously.

Exotic Newcastle disease is a contagious and fatal viral disease affecting all species of birds and is probably one of the most infectious diseases of poultry in the world. It is endemic in many countries of Asia, the Middle East, Africa and Central and South America. Some European countries are considered free of Newcastle disease. Genetic variation exists between birds in their ability to cope with infection, specifically in their mortality post-infection (Gordon et al. 1971). There is also genetic variation in antibody responses to Newcastle disease virus (NDV) vaccines in turkeys (Sacco et al. 1994) and quantitative trait loci (QTL) for antibody responses to NDV have been located (Yonash et al. 2001). Lines of turkeys previously selected for various performance traits differed in their Newcastle disease mortality (Tsai et al. 1992), with increased meat production apparently being adversely correlated with survival and large breed differences being reported in mortality rate following challenge with NDV (Hassan et al. 2004). Thus, it should be possible to select for resistance to Newcastle disease, although considerable refinements need to be made before it becomes a practical reality. This selection may be of limited use in the USA or in western Europe where the

disease is successfully addressed by eradication strategies. However, it may be of immense importance in countries where the disease is endemic, as it is in Africa and Asia.

Tuberculosis (TB) is a contagious disease of importance for both animals and humans. Bovine TB, caused by *Mycobacterium bovis*, can be transmitted from livestock to humans and other animals. A number of wild animals (European badger, the brush-tailed possum, buffalo, bison and several species of wild deer) can act as reservoirs of *M. bovis* for cattle and other mammals, and these pose the major impediments to disease control by means of eradication. Genetic solutions for controlling TB transmission are increasingly attractive, because they offer the chance of reducing TB prevalence, even in the presence of a reservoir of infection.

Genetic variation in TB resistance is reasonably well established, with documented differences between breeds and families under field conditions, as well as an implied involvement of the *Nramp1* gene (Adams and Templeton 1998). Further evidence that alleles at *Nramp1* affect susceptibility to TB, but not disease progression once animals are infected, is given by Zanotti et al. (2002). In deer studies in New Zealand, TB resistance has been shown to be strongly heritable ($h^2 = 0.48 \pm 0.09$) (MacKintosh et al. 2000). In humans, analysis of twin and familial data has demonstrated genetic variation in resistance to infection (cited by Adams and Templeton 1998). The mouse equivalent of *Nramp1*, i.e. *Bcg*, has been shown to control mycobacterium infections (cited by Adams and Templeton 1998).

Thus, TB is a disease for which enhanced host resistance is likely to contribute to sustainable disease control. However, further research is required to identify the loci and/or genes responsible for variation in susceptibility in cattle and other farmed animal species.

Trypanosomosis is an important constraint to livestock production in tropical Africa. Trypanosomes are borne by tsetse flies. Kristjanson et al. (1999) estimated that at least 46 million cattle are kept in tsetse-infested environments, of which 17 million are treated with medication at an annual cost of \$35 million. Total annual costs, including production losses, mortality and reduced fertility, are estimated to exceed \$1 billion (Falconi et al. 2001). A large international research effort aimed at detecting genes conferring tolerance of or resistance to this infection has revealed 10 trypanotolerance QTL on 9 chromosomes (Hannotte et al. 2003). This information was obtained from the crossbred progeny of trypanotolerant and susceptible breeds. Interestingly, the trypanotolerant allele came from the resistant N'Dama breed in five cases and the more susceptible Boran breed in four cases (so-called 'cryptic' genes). Further research is underway to identify the genes responsible within these QTL and/or genetic markers that would facilitate selective breeding for trypanotolerance.

Transmissible spongiform encephalopathies (TSEs) are fatal degenerative diseases of the central nervous system that affect many mammalian species and include bovine spongiform encephalopathy (BSE) in cattle, scrapie in sheep and Creutzfeldt-Jakob disease (CJD) in humans. Scrapie has been

endemic in some European sheep populations for more than 250 years. Features of TSEs are long asymptomatic incubation periods, with death typically occurring several months or years after infection. The infectious causal agent for TSEs is not as yet defined and is still the topic of controversy. However, the prion protein (PrP) has been implicated and TSE pathology is characterised by the accumulation of wrongly folded PrP, PrP^{Sc}, in lymphoid tissues and the nervous system, especially the brain. Thus, presence of PrP^{Sc} defines the presence of the disease.

In sheep, the presence of scrapie depends jointly on exposure to infection and the genotype of the animal, as determined by the PrP gene. Variation at codons 136, 154 and 171 of the sheep PrP gene jointly determine much of the variation in relative susceptibility to scrapie (Goldmann et al. 1991; Hunter et al. 1991, 1996). The five known alleles in sheep are termed: ARR, AHQ, ARH, ARQ and VRQ and yield 15 PrP genotypes. Characterisation of the implication of the 15 PrP genotypes is given at

www.defra.gov.uk/animalh/bse/othertses/scrapie/nsp/pdf/genotypes.pdf.

Genotypes containing the ARR allele are relatively resistant, and genotypes containing the VRQ allele are susceptible. This apparently simple genetic control of scrapie susceptibility leads to the opportunity to breed sheep for increased scrapie resistance (Dawson et al. 1998). The importance of TSEs has led to the development of breeding programmes in several European countries aimed at reducing and eventually eliminating TSEs from national sheep flocks. Details of the UK National Scrapie Plan, for example, may be found at: www.defra.gov.uk/animalh/bse/othertses/scrapie/nsp/index.html.

Several questions arise about selection on PrP genotype. First, the mechanism of resistance is unknown. We do not know whether PrP genotypes code for relative resistance to infection (or risks of clinical signs of disease), or whether or not they simply influence incubation period, as seen in mouse models (Moore et al. 1998) with possible transmission from asymptomatic carriers. In this latter case, the likely reduction in scrapie incidence resulting from the breeding programme would be slightly reduced compared to the case where PrP genotype coded for resistance to infection. Second, genetic variation for scrapie exists outside the standard PrP alleles. For example, further variability within the PrP gene associated with scrapie has been described, particularly at codon 141 (Moum et al. 2005), and genetic variability in scrapie survival time for sheep of fixed PrP genotype has been described and mapped to regions unlinked to the PrP gene (Moreno et al. 2002). Therefore, there is additional unexploited variation in scrapie resistance. Third, the effect of genotypes in terms of scrapie resistance may not be consistent across breeds. And lastly, possible undesirable relationships between PrP genotype and performance traits are often raised as a risk for PrP selection. However, such adverse impacts on performance traits have yet to be demonstrated.

Goats are also considered to be very susceptible to scrapie, though information here is still largely anecdotal. Although the caprine PrP gene is highly polymorphic, few linkages to scrapie resistance have yet to be convincingly documented (Detwiler and Baylis 2003). There is little evidence

of genetic variation in the bovine PrP gene associated with susceptibility to BSE, but QTL influencing susceptibility have been located (Hernandez-Sanchez et al. 2002; Zhang et al. 2004).

Selective breeding for disease resistance is already being implemented for a number of infectious diseases that do not appear on the OIE's list of notifiable diseases. For example, DNA marker tests are being used to select pigs with resistant genotypes for *E. coli* F18 and F4-induced diarrhoea (Meijerink et al. 2000; Jorgensen et al. 2003). Both tests have been patented.

Host genetics: conclusion

Exploiting host genetic variation in resistance to, or tolerance of, disease already makes a contribution to disease control and has the potential to make a much greater impact. For more detailed reviews of the genetics of disease resistance in farmed animals, see Bishop 2004; Gibson and Bishop 2005. Farm animal genetics and genomics research is now increasingly addressing the genetics of host responses to infectious disease. The outputs from this research will inform new opportunities for selective breeding for disease resistance. In the context of this research, disease resistance attributes possessed by indigenous livestock in Africa and China, and wild related species, may make a major contribution to research aimed at identifying the mechanisms underlying resistance. The maintenance of such livestock resources should be valued highly in order to preserve future options.

Engineering the host genome

Selection is constrained by the genetic variation for the trait of interest and by species boundaries. The traditional means of acquiring new and desirable genetic variants is by cross-breeding, perhaps to exotic stock lines. For example, the genes for prolificacy presumed to be present in Chinese pig breeds, such as the Meishan, have been introduced to European pig stocks by cross-breeding. However, the resulting cross-bred individuals also inherit a proportion of the undesirable features of their exotic parents, such as the high fat content of Meishan meat. Transgenesis (or gene transfer), which brings together recombinant DNA and advances in reproductive technologies, offers the possibility of introducing single new genes, or of modifying genes that are already present, without any unwanted genetic baggage. Transgenesis also allows the movement of genes across species, thus permitting modifications not possible by other methods.

It is 20 years since the first transgenic farm animals were created (Hammer et al. 1985). Subsequently, several significant improvements in transgenic technology and its application to farmed animals have been achieved. However, in addition to issues of public acceptance of genetically modified farm animals, there are a number of technical issues that need to be addressed before transgenesis can compete with selective animal breeding. Modification of the host's genome in order to enhance disease resistance or tolerance, however, represents an area where gene transfer could deliver improvements beyond the reach of conventional selective breeding.

Gene transfer technology

Gene transfer technology as applied to farm animal species is the subject of several authoritative reviews (see Clark and Whitelaw 2003; Niemann et al. 2005). We have therefore restricted our commentary to showing how the different technologies enable or limit gene transfer as a means of modifying the capability of the host to resist or tolerate infection.

Briefly, gene transfer can be achieved by direct injection of DNA, using sperm as a vector, by use of disabled viruses, or by developing animals from cells modified during culture *in vitro*.

DNA (micro)injection

Transgenic animals can be produced by direct microinjection of multiple DNA copies of a gene into single-cell eggs (Hammer et al. 1985; Simons et al. 1988). Although this technique has been used to produce transgenic mice, rabbits, pigs, sheep, cattle and chickens, it suffers from a number of significant problems.

First, less than 5% (often 1% for non-rodents) of the injected eggs develop into transgenic individuals. Second, the chromosomal site, at which the transgene integrates, and the number of tandem copies of the gene that are integrated, cannot be pre-determined. As a result, the expression of the transgene often differs between independent lines. The transgene can cause insertional mutagenesis, and recessive phenotypes are very difficult to detect and assess. The technique is limited to adding new genetic material and cannot alter, in a targeted way, existing genetic material.

The challenges in making transgenic poultry are perhaps greater. When a chicken egg is laid, it contains a developing embryo composed of tens of thousands of cells. Genetic modifications introduced at this stage will generate a mosaic of cells with different genetic changes. The culture system developed at Roslin Institute by Margaret Perry created opportunities to introduce genetic changes in single-cell chicken embryos (Perry 1988; Love et al. 1994).

Sperm-mediated gene transfer

Transgenic pigs have been produced by *in vitro* fertilisation with sperm that had simply been incubated with DNA (Gandolfi et al. 1989; Lavitrano et al. 2002). Although there was initially doubt as to whether this technique could be transferred successfully to other laboratories (Brinster et al. 1989), a recent summary suggests that this is a potentially useable technique in animal transgenesis (Smith and Spadafora 2005). However, the final fate of the exogenous sequences transferred by sperm is not always predictable. An analysis of animals derived by this means suggested that, while the foreign sequence is sometimes integrated, stable modifications of the genome are often difficult to detect (Smith and Spadafora 2005). The limitations associated with random integration as described for microinjection also apply to sperm-mediated gene transfer.

Viral vectors

Gene transfer is not a domain populated solely by technically advanced humans; there are some natural gene transfer systems. For example, one class of viruses, retroviruses, have the capability to copy their genome into the chromosome(s) of the host that they infect. The retroviral genome can subsequently be excised from the host chromosome, repackaged into an infectious particle and move on to infect another cell/host. As a result of errors in the integration and excision events, DNA picked up from one host can be transferred to a subsequent host. Thus, modified and partially disabled retroviruses could form the basis of a gene transfer system.

Retroviral vectors have been created, which retain the ability to integrate copies of their genomes into host chromosomes, but which have lost the ability to subsequently produce further infective virus. The potential advantages of retroviral gene transfer are that non-surgically recovered multicellular embryos can be infected and integration is usually a single copy event. In species such as poultry, where the single-cell embryo is particularly inaccessible and difficult to handle, retroviral vectors may be especially useful. Bosselman et al. (1989) described the integration of foreign DNA into the germline of chickens following injection of a replication-defective retroviral vector through the area pellucida into the subgerminal cavity of the blastoderm of unincubated eggs. Low levels of competent virus were found in some of the resulting transgenic birds, but not in those selected for breeding. Retroviral vectors have also been used for gene transfer into cattle, sheep and pig embryos (Kim et al. 1993; Hettle et al. 1989; Petters et al. 1988).

The disadvantage of using multicellular embryos is that the transgenic animals produced will be mosaics and the germline may be chimaeric. Transmission of the transgenes, therefore, may vary from the inefficient to the non-existent. Other disadvantages of the use of retroviral vectors are their limited capacity for foreign DNA sequences and the adverse effects of some retroviral sequences on expression of the transgene. Public acceptability may also prove a significant barrier to the use of retroviral vectors for gene transfer in livestock. For example, the remobilisation of an apparently defective provirus reported by Crittenden and Salter (1990) tends to confirm the suspicion that validating the safety of retroviral vector systems may be difficult.

Lentiviral vectors

An important recent development with potential for gene transfer in farm animals concerns lentiviruses (Pfeifer 2004). These are a class of non-oncogenic retroviruses that are being used to develop replication-defective viral vectors for gene therapy (Thomas et al. 2003). Gene transfer using lentiviral vectors has been successfully achieved in cattle (Hofmann et al. 2004), pigs (Hofmann et al. 2003; Whitelaw et al. 2004), chickens (McGrew et al. 2004) and sheep (Whitelaw, personal communication). The main advantage of lentiviral-based gene transfer is its extraordinary efficiency. For example, 92% of the piglets born following infection of single-cell embryos were transgenic and 95% of these transgenic animals expressed the transgene. The main disadvantages are those associated with random

integration (see above), the limited capacity of the vector (<10 Kbp) and issues concerning safety and public acceptance.

Cellular vectors: embryo stem cells, nuclear transfer, gene targeting

The problems with gene transfer by microinjection are two-fold: the uncertain nature of the genetic change effected, and the low frequency of success. Thus, systems that allow the nature of rare successful events to be evaluated *in vitro*, for example, in cultured cells would be attractive. Methods for introducing additional genetic material into animal cells grown in culture had been established several years prior to the generation of transgenic mice. While generating a genetically modified animal cell in culture may be relatively straightforward, creating an entire animal from the resulting cell(s) is not.

Although liver, kidney or white blood cells grown in culture may be genetically identical to the fertilised zygote that developed into the animal from which the cells were isolated – i.e. they share the same DNA sequence – they have subsequently become specialised or terminally differentiated. As a result of epigenetic changes that have occurred during the development, such differentiated cells have lost their potential to develop into other cell types. Cells that have the potential to develop into every cell type found in an adult are termed 'totipotent'.

In order to pursue a cellular-based approach to gene transfer, it is necessary either to carry out the genetic modification in cells that are totipotent or to reprogramme the nucleus of the modified cell in order to restore its totipotency. These options have been successfully effected using embryo stem cells and nuclear transfer respectively. Cells isolated from the inner cell mass of blastocyst-stage embryos can be cultured *in vitro* under specific conditions that allow the cells to retain their totipotency (Evans and Kauffman, 1981). During the time these embryo-derived stem cells (ES cells) are grown in culture, genetic changes can be effected. In particular, changes that have a low success rate can be attempted, such as homologous recombination between a targeting vector and the host chromosome in order to modify an endogenous gene (Capecchi 1989).

A variety of targeting strategies have been developed in order to either disrupt endogenous genes or to make subtle changes to the endogenous gene. Once ES cells have been modified in culture, they can be incorporated into a reconstituted embryo and transferred to a host female. A proportion of the resulting progeny may contain cells descended from the modified ES cells, leading to founder transgenic animals. However, it must be recognised that these transgenic animals are chimaeric, as a result of mixing totipotent cells from multiple sources. Thus, the ES-cell route to genetic manipulation has limitations due to uncertainties concerning germline transmission to subsequent generations.

The development of totipotent embryo stem cells in mice opened the door to engineering precise genetic changes to the mouse genome. In the past 10–15 years, hundreds of genes have been subjected to targeted modification in mice, though most of the modifications have involved disrupting the relevant

locus (knockouts). Details of many of these transgenic mice are stored in TBASE: the Transgenic/Targeted Mutation Database (<http://tbase.jax.org/>). Despite concerted efforts by a number of laboratories from the late 1980s onwards, no one, as far as we are aware, has isolated embryo stem cells from farm animal species *and* demonstrated their totipotency.

When reviewing the prospects for gene transfer in farm animals about 12 years ago, one us (ALA) concluded that, 'for sheep and cattle, therefore, ES cells in conjunction with nuclear transfer would represent an ideal gene transfer system.' This conclusion was based on the assumption that totipotent ES cells for farm animals would emerge from the laboratories reporting the isolation of cells from sheep and pigs with some ES-cell-like characteristics. It was known that nuclei from sheep inner-cell masses, unlike their mouse counterparts, were capable of supporting normal development after transfer into enucleated oocytes (Smith and Wilmut 1989).

The key breakthrough in developing a cellular route for gene transfer in farm animals was the cloning of sheep by nuclear transfer where the donor nuclei were from cells cultured *in vitro* (Campbell et al. 1996; Wilmut et al. 1997). Although the production of 'Morag' and 'Megan' established the principle, it was 'Dolly' the sheep, the product of nuclear transfer from a cultured somatic cell (from an adult mammary gland) into an enucleated oocyte (Wilmut et al. 1997), that captured the headlines. Nuclear transfer cloning technology has been extended to other species including cattle and pigs (Polejaeva et al. 2000; De Sousa et al. 2002). The donor nuclei in these experiments were not genetically modified prior to transfer.

The next step in the development of a cell-based method for gene transfer in farm animals involved the creation of a sheep called 'Polly' by nuclear transfer, where the donor nucleus was from cells that had been genetically modified by the addition of a human factor IX gene (Schnieke et al. 1997). Finally, the production of sheep with targeted genetic modifications, including deletions of specific genes, has been achieved using nuclear transfer (McCreath et al. 2000; Denning et al. 2001). Cloned pigs have also been produced by nuclear transfer using nuclei from cells, in which targeted genetic changes have been made (Dai et al. 2002, Lai et al. 2002).

Cellular routes to gene transfer in farmed mammals have addressed the requirement for precision: directed genetic changes can be confirmed *in vitro* prior to creating a genetically modified animal. However, the nuclear transfer elements of the method are profoundly inefficient.

Types of genetic modification

All the gene transfer methods described above can be used to introduce additional genetic material into the genomes and germlines of animals. There are limits on the size of the additional DNA fragments that can be introduced using viral vectors. Fragments of 100,000 to 500,000 bp or more, however, can be introduced using direct microinjection. If direct modification of the genomes of farm animals is contemplated, then adding new or extra copies of genes represents only one class of change that might be considered

beneficial. A range of modifications to endogenous genes might be desirable including: knockouts; changing in coding or regulatory sequences; and increasing or decreasing the level of expression.

Targeted changes to an endogenous gene, including changes in its sequences, rely on very rare homologous recombination events and can therefore only realistically be achieved by the cellular route. Gene addition, which is deliverable by all the systems described, however, can be used to effect reductions in the expression of endogenous genes.

RNA interference is a phenomenon in which double-stranded RNA (dsRNA) silences genes that share sequence homology with the dsRNA. Short interfering RNAs (siRNA) are key intermediates in gene silencing by RNA interference. It has been demonstrated that siRNAs can be used to silence genes in animal cells (McManus and Sharp 2002). Gene silencing has been demonstrated in mice carrying transgenes that are transcribed to yield siRNAs (Tiscornia et al. 2003) or short-hairpin RNAs (shRNAs) (Rubinson et al. 2003).

Engineering the host genome for resistance to infectious disease

Gene transfer technology offers opportunities to add novel genes to the genome of a host to increase its ability to resist or tolerate infection. Transgenes for gene addition can be rare alleles in the target species, sourced from another species, or they may represent completely novel, man-made constructs. For example, one of the early farm animal transgenic experiments involved adding the mouse Mx1 gene, which confers resistance to influenza, to pigs (Muller et al. 1992). Unfortunately, the resulting transgenic pigs were not tested for resistance to influenza.

Genes whose products attack or neutralise the pathogen could represent valuable additions to the host genome. Mice suckling from transgenic mice expressing neutralising antibodies to murine hepatitis virus were protected from MHV-induced encephalitis (Kolb et al. 2001). Transgenic cows expressing lysostaphin, an endopeptidase that enzymatically disrupts the cell walls of *Staphylococcus aureus* (the major pathogen responsible for mastitis), exhibit increased resistance to *S. aureus* infections (Wall et al. 2005).

More generally, genes that confer a dominant negative phenotype would be candidates for gene addition. For example, it may be possible to block infection by specific viral pathogens by interfering with the interaction between the virus and host molecules that act as receptors for viral entry. This approach has been exemplified in a mouse model of pseudorabies-resistant livestock in which the transgenic mice express a soluble form of the nectin-1 protein that facilitates entry of pseudorabies virus (Ono et al. 2004). All of the transgenic mice were almost completely resistant to infection with pseudorabies virus through either an intra-peritoneal or intra-nasal route. The mechanism through which resistance was achieved is unknown but could include binding between the soluble nectin-1 protein and the virus or virus glycoproteins.

Host genes relevant to infectious disease that could be targets for gene knockout or knockdown would include those encoding receptors for pathogens. For example, as discussed above, variation in the FUT1 (Meijerink et al. 2000) and MUC4 genes are associated with susceptibility/resistance to *E. coli* F18 and F4 respectively in pigs. Removing these genes or attenuating their expression could confer resistance on the resulting transgenic animals. Aminopeptidase N has been identified as the receptor for porcine transmissible gastroenteritis virus (TGEV) (Schwegmann-Wessels et al. 2002) and reducing the expression of the host gene encoding this protein might render the transgenic pigs resistant to TGEV. Similarly, sialoadhesin facilitates internalisation of the porcine reproductive and respiratory syndrome virus (PRRSV) (Deputte and Nauwynck 2004) and, again, knocking down expression of this gene might enhance the pig's ability to resist infection with PRRSV. As these host genes presumably did not evolve to facilitate infection by pathogens, knocking out these genes or reducing their expression may have deleterious side-effects for the host.

The gene-encoding prion protein is also an obvious target for gene knockout technology in food animal species, given its key role in the transmissible spongiform encephalopathies. Transgenic mice in which both copies of the PrP gene have been disrupted are resistant to scrapie (Prusiner et al. 1993). Both sheep and cattle with PrP gene knockouts have been created (Denning et al. 2001; Kuroiwa et al. 2004). Unfortunately, the founder transgenic lamb had developmental problems and was euthanised, and details of the phenotypes of transgenic cattle lacking functional PrP genes are not yet in the public domain.

The targets for an RNAi transgene need not be limited to host genes. In the context of infectious disease, the targets could include pathogen genes and transcripts. This approach is particularly attractive where the pathogen is a virus, such as the RNA viruses that constitute over two-thirds of the OIE list-A pathogens. The potential of interfering RNAs to inhibit foot-and-mouth disease (FMD) viral replication has been demonstrated in BHK-21 cells and in a suckling mouse model (e.g. Mohapatra et al. 2005; Chen et al. 2004). Colleagues at Roslin Institute and others are working on creating transgenic farm animals and mouse models expressing RNAi transgenes targeted against pathogens. As far as we are aware, as yet no one has reported achieving this objective. However, RNAi is being considered as a potential solution to infectious diseases such as FMD (Grubman and de los Santos 2005) in livestock and for many other diseases in livestock, aquatic and plant host species..

Engineering the host genome: conclusion

Genetic modification of the host genome offers opportunities to enhance the ability of farmed animal species to resist or tolerate infectious disease. In principle, this leads to new methods of disease control that are not reliant on existing genetic variation in the host population. In particular, engineering hosts to knockdown the expression of viral genes and inhibit viral replication is an attractive option. A particular example of relevance to this review relates to

avian influenza, perhaps the major livestock disease threat in China – with worldwide implications. In this case, the possibility exists, and is being investigated by colleagues at Roslin, of using RNA interference techniques to block viral infection. A particular advantage of genetic modification would be the ability to engineer a suite of disease-resistance genes into a single insertion. It may also be possible to create disease resistance based on a pathway of genes, an application of which may be the engineering of animals to express a plant product. These are applications that would be difficult and often impossible to achieve by conventional breeding techniques.

Further improvements in genetic modification technology are required before it is cost-effective. However, it should be recognised that all technologies incur substantial costs in their development. Such improvements are likely in the next 10–25 years, during which time we are also likely to see many more experiments that will prove the principle that genetic modification can contribute to disease control strategies in farmed animal species.

Public resistance to the genetic modification of crops and farm animals is likely to remain an issue. However, genetically modified animals that reduce the risk of infection, and, in particular, zoonotic infections, may be more readily accepted than other modifications. Such changes may have particular value in the developing world, where relative costs of disease are higher than in the UK, where other disease control measures are often difficult to implement and sustain, and where failure of disease control can have devastating effects on the lives of resource-poor farmers. In line with this, the Science Council of the CGIAR has recently (October 2005) ratified a strategic report with priorities for action, recommending that the CGIAR convene expert groups on the potential for genetic modification of livestock in the developing world (Gibson 2005). Disease resistance is likely to be the top priority for genetic modification. It would be timely for the UK to team up with the CGIAR to explore this area and to review options for genetic modification to increase disease resistance.

Critique: can host genetics or engineering contribute to the detection and identification of infectious disease?

There is clearly a role for selective breeding for disease resistance and, potentially, for genetically modified, disease-resistant livestock in strategies to control infectious diseases in farmed animal species in the next 10–25 years.

We consider that any contribution of host genetics or engineering of the host genome to the detection and identification of infectious disease is much more speculative. However, there are potential options in terms of sentinel animals or plants, as described below.

The rationale for exploiting host genetics and genetic modification in disease control strategies is to develop resistant or tolerant genotypes. Such genotypes not only have the potential to enhance productivity in the face of pathogen challenges and hence the sustainability of animal production systems, but also to reduce the risk of epidemics. Depending on the infectiousness of the disease, a relatively modest proportion of resistant

individuals can significantly reduce the risk of an epidemic (Bishop and MacKenzie 2003). Conversely, for highly infectious diseases, a very high proportion of resistant animals may be required before other disease control measures such as vaccination can be reduced. Where the selected genotypes have enhanced tolerance rather than resistance, there are risks that reservoirs of the pathogen(s) will be maintained while the animals are asymptomatic. There are self-evident risks to the human population in selecting for tolerant animal genotypes when the disease concerned is also zoonotic.

For disease detection and identification, it is desirable to have assays, including the bio-assays represented by whole animals, that reveal the presence of the disease and its pathogen in a timely manner. Thus, the genotypes (natural or engineered) that are desirable for disease control are ill suited as early indicators of the presence of a disease challenge. What is required for the detection and identification of infectious disease is sentinel animals or animals that can signal the risk in a manner equivalent to canaries in a gas-contaminated mine. There are several options for this approach.

It would be possible to design genetic strategies to identify or create susceptible genotypes or transgenic animals with increased susceptibility to a specific disease (or diseases). However, if the pathogens were free to multiply in such animals, then, in addition to being an early-warning system, the sentinels might represent a risk to the wider population. This is a risk that would need to be carefully managed.

The resistant genotypes discussed above are generally specific to one of a limited number of pathogens. There is therefore considerable interest in identifying genotypes associated with generalised immunity or resistance to infection. Much current research addressing this strategic goal is examining the role of the innate immune system and key early detectors of pathogens such as the Toll-like receptors. Similarly, in order to have utility, any sentinel genotype should be able to signal the presence of a wide range of infectious disease challenges. If developing such sentinel genotypes were considered appropriate for detecting infectious disease, again, the innate immune system and early detectors of pathogens would be a natural focus of attention. For example, the analysis of a range of gene expression data from disease challenge experiments is revealing patterns of changes in gene expression that are common to several infections (Jenner and Young 2005). Selective breeding or genetic modification to 'turn up the volume' on these signals or to link these changes in gene expression to a simple reporter system (e.g. a fluorescent or other readily detected molecule excreted in urine or faeces) might allow the concept of a sentinel animal to become a practical reality.

Similarly, sentinel animals may (conceptually) be engineered through reporter systems that use non-immune-based evidence of infection or pathology. For example, engineered cells in the gut, liver or lungs may report excess breakdown metabolites indicative of pathology and, hence, the presence of a particular disease or disease category. In all these cases, parallel systems and examples may be constructed for plants, with the delivery of the technology perhaps being easier than it is for animals.

However, even in the situation where it is technically possible to produce sentinel animals, the logistics of utilising them are difficult. To be useful as sentinels, such animals would have to be widespread within a livestock production system. This would require a logistically well-organised breeding and distribution programme, as well as a sophisticated monitoring system. Additionally, the interpretation of the sentinel animal signals has to be timely, so that the signal is given early enough and acted on quickly enough to enable effective disease management decisions to be taken.

We also consider that the value of such signalling sentinels may be limited compared to the vigilance of animal handlers and veterinarians. The exception might be for animals that are allowed to roam free for long periods, or in cases where current diagnostic techniques are slow or difficult. However, even this situation requires close scrutiny, as the prevalence of infection with such diseases must be considered. For example, TSE infections may at first sight lend themselves to the use of sentinel animals, as diagnosis is both slow (clinical signs may take several years to be apparent) and difficult. But, in this case, the within-herd prevalence of infection is so low that the probability of the sentinel animal becoming infected is small, even if other infected animals do exist in the herd.

A further risk that can't be ignored is that of pathogen evolution, particularly with viral infections where evolution rates are often fast. The specific risk is that animals may be engineered to respond to a particular viral attribute, but mutations in the virus may result in them not being efficiently recognised or dealt with by mechanisms in the engineered animal. Pathogen evolution is a potential drawback with all disease control strategies, with risks minimised by avoiding reliance on single mechanisms of disease control. The same principle should also apply to disease detection strategies, insofar as the detection methods must be robust and able to detect different strains of the same pathogens.

In contrast to the animal situation, such sentinel individuals may have utility in disease detection in crop plants. In this case, many of the logistical issues are simpler, the engineering techniques are more advanced, and the nature of the diseases may lend themselves better so such technologies.

In conclusion, we consider that, although host genetics and engineering have important contributions to make to disease control, any contribution to the detection and identification of infectious disease is speculative, far into the future, and possibly fanciful. However, in reviewing the continued output from research on host–pathogen interactions and the genetic control of host responses to infectious disease, we argue that there is a role for imaginative tangential thinking that could include consideration of sentinels and canaries.

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Authors – details of relevant experience, capabilities and knowledge

Professor Alan L. Archibald FRSE

Professor Archibald has over 30 years' experience in research on farmed animal species, principally pigs and ruminants (cattle and sheep). Although his first degree is in biochemistry, most of his research has been within the disciplines of genetics, molecular genetics and genomics. He led the first international collaborative farm animal genomics project (PiGMaP) and the team that established the first genome database for a farm animal (PiGBASE). His current research projects include studies on the genetics of host responses to porcine reproduction and respiratory syndrome virus (PRRSv) and trypanotolerance in cattle. He contributed to the discovery of genetic markers that are predictive of susceptibility to *E. coli* F4-induced diarrhoea in pigs. He also contributed to the first elucidation of the molecular genetic lesion underlying a regulatory QTL in farm animals. He is a member of the leadership of the Swine Genome Sequencing Consortium, which is developing a programme to sequence the pig genome. He also has experience of genetic modification in farmed animals, including being part of the team that developed transgenic sheep for the production of human pharmaceutical proteins in milk and the team that cloned a pig using the 'Dolly' technology. He is an Honorary Professor in the Royal (Dick) School of Veterinary Sciences at the University of Edinburgh and has recently been elected a Fellow of the Royal Society of Edinburgh.

Professor Stephen C. Bishop

Steve Bishop is a Senior Principal Investigator at Roslin Institute, where he leads the disease genetics programme. He is also a leader of the European Animal Disease Genomics Network of Excellence (EADGENE), a Special Professor at Nottingham University, and was a Visiting Professor at Glasgow Veterinary School (1998–2001). He has 20 years' experience of strategic and applied research in animal breeding and genetics, with a major focus on disease genetics in the last 10 years, including topics as diverse as ruminant nematode infections, PRRS and Salmon IPN. He has also led the development of theory and modelling techniques combining genetics and epidemiology. He was commissioned to co-edit the second edition of *Breeding for Disease Resistance in Farm Animals* (CAB International 2000), was invited to prepare the article on disease genetics in the *Encyclopedia of Animal Science* (2004), and he led the development of the Food and Agriculture Organisation (FAO) disease genetics strategy, resulting in the document, *Opportunities for Incorporating Genetic Elements into the Management of Farm Animal Diseases: Policy Issues* (www.fao.org/ag/magazine/bsp18-e.pdf).

Table 1: Examples of diseases for which there is documented or strong anecdotal evidence of genetic variation in host resistance or tolerance

Host species	Pathogen or parasite type			
	Prion and virus	Bacteria	Protozoa	Helminth and ectoparasite
Chickens	Marek's disease	E. coli	Coccidiosis	Ascaridia galli
	Infectious laryngotracheitis	Pullorum		
	Avian leucosis	Fowl typhoid		
	Infectious bursal disease	Salmonellosis		
	Avian infectious bronchitis	Campylobacter		
	Rous sarcoma			
	Newcastle disease			
Pigs	African swine fever	Neonatal diarrhoea		
	Foot-and-mouth disease	Post-weaning diarrhoea		
	Atrophic rhinitis			
	Pseudorabies			
Cattle	BSE	Paratuberculosis	Trypanosomosis	Helminthosis ¹
	Foot-and-mouth disease	Mastitis	Theileria annulata	Ticks
	Bovine leukaemia	Bovine tuberculosis	<i>Theileria segenti</i>	
		Salmonellosis	<i>Theileria parva</i>	
		Dermatophilosis	Babesia	
		Cowdriosis		
		Brucellosis		
Sheep	Scrapie	Footrot	Trypanosomosis	Helminthosis ¹
		Mastitis		Liver fluke
		Paratuberculosis		Flystrike
		Dermatophilosis		
		Salmonellosis		
		Cowdriosis		

1. Host resistance to many species of nematode helminths has been described.

All the reports and papers produced within the Foresight project 'Infectious Diseases: preparing for the future,' may be downloaded from the Foresight website (www.foresight.gov.uk). Requests for hard copies may also be made through this website.

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