POTENTIAL EFFECTS OF BIOTECHNOLOGY ON ANIMAL HEALTH AND WELL-BEING

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Animal disease is a major social and economic problem across the United States, and throughout the world. Diseases can lead to animal suffering and distress, reduced performance, and possibly even death. Infectious diseases have major negative effects on poultry and livestock production, both in terms of economics and on animal welfare. The costs of animal disease are estimated to be 35-50% of turnover within the livestock sector in developing countries, and 17% in the developed world. Often animal disease is fought through vaccination or the use of antibiotics. However, the use of antibiotic in animal agriculture is meeting increased disapproval among consumers. In addition to enhancing animal well-being, improving animal health has the added benefit of reducing the need for veterinary interventions and the use of antibiotics and other medicinal treatments

Animal biotechnology offers a number of approaches to fight disease in animals. Firstly, through genetic selection, livestock producers can select for certain genetic variations which have been associated with disease resistance. Through careful selection, they can develop populations of animals that are less vulnerable to disease. Secondly, through genetic engineering, breeders can integrate disease resistance genes from new sources, allowing for improved animal health. Disease resistance benefits not only livestock producers and their animals, but consumers also benefit as a result of safer animal products in the market place, and a reduction in the incidence of human-transmissible diseases such as avian influenza.

Fig. 1. Beef cattle grazing. Cattle are one species that could benefit from selection for disease resistance. Major diseases affecting cattle include foot and mouth disease, mad cow disease, mastitis, shipping fever, and brucellosis. Photo by Bill Pohlmeier.

Disease Resistance through Genetic Selection

One method to increase disease resistance in a population of animals is to select animals which show resistance to a disease to be the parents of future generations. In this way, animals with specific genetic variations associated with disease resistance can pass those genetics on to their offspring, thus increasing the likelihood that their offspring will be resistant to infection. It has long been known that mice carrying a certain version (allele) of the Mx gene show resistance to influenza infection[1], with the certain allele responsible for resistance known as Mx1[2]. A homologous, or similar, Mx gene has been identified in swine[3]. *In vitro* studies reveal that certain forms of the swine Mx gene confer different levels of resistance to influenza infection[4], and it is thought that future *in vivo* studies will reveal whether selection for a certain form of swine Mx gene results in influenza resistance within a swine population.

Fig. 2. Sow with piglets. Major diseases affecting pigs include foot and mouth disease, influenza, swine fever, respiratory and wasting diseases. Photo courtesy of Agricultural Research Service, USDA.

Chickens are a natural host for avian influenza. As such, influenza infection in chickens can lead to sickness and even death. As with mice and swine, an Mx gene has been identified in chickens[5, 6]. *In vitro* studies show that certain copies of the chicken Mx gene display different levels of antiviral activity[7], suggesting that certain populations of chickens might be less susceptible to avian influenza infection, based on their genetic background. However, similar to swine, *in vivo* studies will be required to determine whether live chickens actually display resistance to influenza infection based on their copy of Mx.

Fig. 3. Chicken is another species that could benefit from increased disease resistance. Major diseases affecting chickens include avian influenza, Newcastle, and Marek's diseases. Photo from animalscience.ucdavis.edu

Disease-resistant Genetically Engineered Animals

While selection for disease resistance in livestock species might prove to be a powerful tool for livestock producers, it is limited by the natural genetic variation present in the species being selected. Genetic engineering, on the other hand, allows for the introduction of novel genetic sequences into the species of interest and is not limited by species barriers as the genetic building blocks in all species are the same. The use of genetic engineering has long been suggested as a method to increase disease resistance in livestock[8], and scientists are currently working on a number of different animal models which could be used to help livestock resist infection (Table 1).

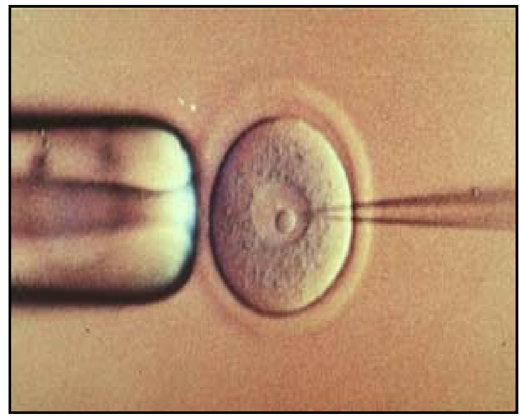

Fig. 4. DNA microinjection. Photo from University of California - Irvine

EXTANT APPLICATIONS	**Species**	**Gene**	**Approach**	**Reference**
BSE resistance	Cattle, Sheep, Goats	Prion	Knockout	9-12
Mastitis resistance	Cattle	Lysostaphin	Transgene overexpression	13
Mastitis resistance	Cattle	Lactoferrin	Transgene overexpression	14
BSE resistance	Goat	Prion	RNAi transgene	15
Visna virus resistance	Sheep	Visna virus envelope gene	Transgene overexpression	16
Mastitis resistance	Goats	Lysozyme	Transgene overexpression	17, 18
GCH virus resistance	Grass Carp	Lactoferrin	Transgene overexpression	19
Bacterial resistance	Channel Catfish	Cecropin B gene	Transgene overexpression	20
ENVISIONED APPICATIONS	**Species**	**Gene**	**Proposed Approach**	
Suppressing infectious pathogens	Various	RNA viruses (eg .foot and mouth, fowl plague, swine fever)	RNAi	21, 22
Coronavirus-resistance	Swine	Aminopeptidase N	RNAi /Knockout	23
Avian flu resistance	Poultry	Avian influenza	RNAi	24, 25
Brucellosis resistance	Cattle	NRAMP1	Transgene overexpression	26

Table 1. Extant and envisioned application for the production of disease-resistant genetically engineered livestock.

Spongiform encephalopathies, such as scrapie in sheep, and Bovine Spongiform Encephalopathy (BSE) also known as "Mad Cow Disease" in cattle are neurodegenerative diseases caused by the misfolding of a prion protein. BSE has been linked to the human neurodegenerative disease called variant Creutzfeldt-Jakob disease[27, 28] and is thus a potential threat to human health. In a procedure known as gene knockout, scientists can target a specific gene in an organism and remove it. This technology was successfully used to 'knockout' the gene which codes for the prion protein in goats[9], sheep[10], and cattle[11, 12]. In addition to generating livestock which are free from the threat of spongiform encephalopathies, these animals could be used as a 'prion-free' source of biological products for use in human medicine. Transgenic goats carrying lentivectors that express siRNAs against the prion protein have been reported[15]. RNAi is a sequence-specific method to selectively knock down endogenous gene expression. It works by introducing transgenic homologous double-stranded gene constructs which enable the stable expression of small interfering (si)RNAs that constitutively suppress target gene expression[29].

Fig. 5. Prion-free sheep. Photo from Denning et al. (2001)

This RNAi This approach may be a highly efficient approach to generate GE animals with targeted gene knockouts in the future, including GE animals that can knockdown infections caused by important contagious RNA viruses such as foot and mouth disease, classic swine fever, and fowl plague [21].

Mastitis, or the infection of the mammary gland, is a disease which costs the US Dairy industry ~$2 Billion a year[30]. In an effort to reduce the effect of mastitis, scientists have developed genetically engineered cattle which are resistant to mastitis infection[13]. These cattle contain a transgene which encodes for the protein lysostaphin, which cleaves the cell wall of the bacteria which cause mastitis. Transgenic cattle produce the lysostaphin transgene in their mammary gland, which then breaks down infectious bacteria present within the gland and results in a healthier mammary gland. Similar studies using a human lysozyme transgene have demonstrated that the direction of transgene expression to the mammary gland in goats can result in reduced mastitis-causing bacteria[17].

Fig. 6. Mastitis resistant dairy cow. Photo courtesy of Agricultural Research Service, USDA.

The benefits of genetically engineered animals with disease resistance are two-fold. Firstly, they result in a healthier mammary gland, thus reducing pain and discomfort in the animals. Secondly, with reduced mammary infection, the milk, and milk products from these animals are going to be healthier, as harmful bacteria are reduced.

Research using genetic engineering to improve disease resistance is also being conducted on aquaculture species. Transgenic catfish have been developed which contain the cecropin B gene from the moth *Hyalophora cecropia*[20]. Cecropin is a small molecule that has shown anti-microbial properties, specifically against many of the bacteria that are harmful to catfish. When transgenic catfish were challenged with a form of E coli, statistically more transgenic fish survived than their non-transgenic counterparts, suggesting that expression of the transgene does indeed confer disease resistance.

A disease with the potential to be treated through genetic engineering is brucellosis. Brucellosis, caused by the Brucella bacteria, is a zoonotic disease, meaning it can be ready passed between animals of different species. The animal population in and around the Yellowstone region in northwest Wyoming provides a prime example of how easily brucellosis can be spread. A number of large animal species (American Bison, Elk, etc.) in this area suffer from brucellosis. When these infected animals come into contact with grazing cattle, the disease is readily passed. Cattle infected with brucellosis often suffer abortions, reduced fertility, and decreased milk production. In addition, brucellosis can also be passed to humans (known as undulant fever) who come into contact with infected animals, and the results can be severe. Recently a link has been established between brucellosis resistance and a variant of the bovine NRAMP1 gene[26] Through genetic engineering, cattle could be produced which highly express the disease resistant version of NRAMP1, thus increasing their resistance to brucellosis.

A major goal of livestock and poultry breeding programs continues to be the identification of disease resistance genes, and genes that enhance immune response. There are a variety of animal biotechnologies that could be used to assist in the pursuit of this goal. Genomic selection and advanced breeding programs provide methods to identify naturally-occurring variation in disease-resistance attributes, while genetic engineering provides an approach to introduce new sources of disease resistance genes into populations. Collectively these technologies have the potential to align animal production systems with sustainability goals such as improved animal well-being due to lower disease incidence, reduced human health risk from zoonotic diseases (e.g. Mad Cow Disease, Avian Influenza, Brucellosis), and the production of safer food due to the decreased use of antibiotics and other medicinal treatments

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