# Genetically Modified Organisms: Basic Technology, Problems and Promises*

**Robert A. Lindberg,** *PhD*[1]; **Paul V. Phibbs,** *PhD*[2]
[1]*North Carolina Biotechnology Center, Research Triangle Park, NC 27709 (corresponding author)*
[2]*Department of Microbiology and Immunology, Brody School of Medicine, East Carolina University, Greenville, NC 27858*



## Abstract

Tremendous advances in the life sciences have paved the way for the development of a powerful array of new biotechnologies. As a consequence, the promise of the genetic manipulation of food animals for social benefit is now becoming a reality. This paper is intended to provide the bovine practitioner with a brief introduction to biotechnology, genomics, bioinformatics, gene mapping, cloning, genetically modified organisms and transgenic animals. Relevant recent advances are discussed in light of their potential impact on the beef, dairy and veterinary industries. The paper concludes with discussions of the distribution of regulatory responsibilities and the challenges facing the widespread adoption of these technologies in food production.

## Résumé

Des progrès remarquables dans les sciences de la vie ont pavé la voie au développement d'une multitude de nouvelles biotechnologies. Par conséquent, la promesse d'une manipulation génétique des animaux de consommation pour le bien de la société devient maintenant une réalité. Cette présentation veut familiariser les vétérinaires en pratique bovine aux concepts de la biotechnologie, de la génétique, de la bioinformatique, de la cartographie des gènes, des organismes modifiés génétiquement et des animaux transgéniques. Les découvertes récentes les plus pertinentes sont discutées à la lumière de leur impact sur l'industrie laitière, la production animale et la médecine vétérinaire. L'article se termine avec une discussion sur l'attribution des responsabilités de réglementation et sur les défis qu'amènent l'adoption à grande échelle de ces nouvelles technologies dans la production d'aliments.

## Genetics, Genomics and Proteomics

Genetics is the study of heritability and the associated analysis of individual gene location, structure and function. The recognition of inheritance patterns (as later codified by Mendel) might be considered the basis for the first genetic modification of organisms, insofar as the understanding of inherited traits has been used for thousands of years to select for optimal expression of desirable traits in plants and animals. With the advent of molecular genetics, the study of gene expression (*i.e.*, protein production based on the template provided by genetic DNA) and its regulation have tremendously expanded our understanding of the relationship between genotype (genetic identity) and phenotype (outward realization of that genetic makeup) for many genes.

The term "genome" was first proposed in 1920 to denote the totality of all genes on all chromosomes within a cell. In contrast to genetics, genomics is the study of the structure and function of the total genetic information stored in the chromosomes of an organism. This would include studies of DNA sequence organization, gene organization, gene expression analysis (*i.e.*, functional genomics) and the similarities and differences in genomic organization and function between different species (*i.e.*, comparative genomics). Further, genomics includes the study of the integrated response of the collective genome to physiologic stimulation or pathophysiologic challenge. Similarly, proteomics is the study of the entire protein complement or "protein universe" of a cell. Proteomics shares with genomics a similar whole organism view; proteomics aims to determine the structure and function of all expressed proteins in a cell, as well as the effect of physiologic or pathophysiologic stimuli on the profile of expressed proteins.

## Bioinformatics

The mammalian genome (whether human, bovine or other) consists of 3 billion nucleotide base pairs of DNA contained in chromosomes, each of which contains thousands of genes. The study, manipulation, and subsequent phenotypic characterization of an entity as enormous and complex as the genome of an organism requires previously unimaginable data management capability. Fortuitously, the growth of information technologies has

*Presented at the 33rd Annual Conference of the American Association of Bovine Practitioners, September 21, 2000, Rapid City, SD.*

paralleled that of the genomics revolution. Thus, emerged the field of bioinformatics, which addresses the collection, organization, and analysis of large amounts of biological data, using computers and databases.

### Gene Mapping

The mapping of the genes to specific locations on human and other genomes has been an area of great attention and activity. An understanding of genomic regulation requires knowledge of the chromosomal location of a family of genes, as well as their relative location on the chromosome. Once this is accomplished for a given gene, one can begin to appreciate genetic variation (polymorphism) amongst individuals, *i.e.,* differences in DNA sequence for the same gene in different individuals. Characterization of single nucleotide polymorphisms (SNPs), which are differences in a single base-pair within a gene, may determine which such variations can be tolerated without affecting protein structure and/or function, and whether the alteration is likely to ultimately be innocuous, beneficial or detrimental.

### Human vs. Bovine Genomes

Comparative genomics (*i.e.,* interspecies genome comparisons) promises tremendous commercial reward for human and animal healthcare and food industries. A recent joint comparison mapping study from the laboratories of Harris Lewin (University of Illinois) and James Womack (Texas A&M) reports remarkable similarity in the bovine and human genomes.[1] The authors found that 83% of the known bovine genes are identical to human genes and that four human and bovine chromosomes may be identical. This suggests an enormous predictive power, in that the map of one species can be used to identify genes controlling important traits in the other (*e.g.*, lactation, reproduction, resistance to infectious diseases). Such information can be used to develop new genetic markers for production traits in cattle, as well as new drug targets for human healthcare and veterinary markets.

## Biotechnologies Relevant to the Beef, Dairy and Veterinary Industries

In its simplest terms, biotechnology is defined as a collection of technologies that use living cells, their molecules or processes to make products or solve problems. Numerous applications of biotechnologies have been designed and implemented to meet the needs of the beef and dairy industries. Already, biotechnology-derived tests for early detection of pregnancy and diagnosis of brucellosis are in use, as is the administration of recombinant bovine somatotrophin (bST). Many biotechnology-based strategies are being studied to maximize the nutrient content of animal feeds, improve metabolic utilization of feeds, minimize nutrients lost in animal waste, develop non-surgical means of sterilization (immunocastration), maximize growth, improve milk and carcass quality, and confer resistance to disease and adverse environmental conditions.[4] In turn, the application of genomics in biotechnology will yield tremendous opportunities for advances related to animal health, the beef and dairy industries, and the use of animals for the generation of human therapeutics.

### Bovine Somatotropin (bST/BGH)

The first commercial bovine application of biotechnology was the development of recombinant bovine somatotropin (bST) or growth hormone (BGH) to supplement endogenous somatotropin (ST). This development was based on the recognition of the role of endocrine (hormonal) regulation of metabolism, growth and lactation. In lactating cows, endogenous circulating ST has been determined to (i) increase secretory activity, blood flow, nutrient uptake and milk synthesis in the mammary tissues, (ii) enhance glucose production in the liver, (iii) promote adipose-related metabolic pathways resulting in increased mobilization of fat stores as required for increased milk production, (iv) decrease muscle uptake of glucose while leaving the responsiveness of insulin and glucagon production by the pancreas unaltered, and (v) promote metabolic pathways resulting in enhanced calcium production.[2] bST was introduced commercially in early 1994 as Posilac®, to be administered repeatedly in an exogenous sustained-release formulation. Table 1 lists potential endocrine strategies (as proposed by Etherton[10]) as alternatives to direct bST supplementation. These strategies involve direct manipulation of the hormonal tissue or circulating concentration, indirect modulation of the biological potency of endogenous ST, and the genetic engineering of cattle or other farm animals to alter or make more uniform the expression of relevant genes and their associated proteins.

It is worth noting that the USDA Office of Technology Assessment, in a paper on biotechnology and the dairy industry, cautioned that "the bST-supplemented cow presents the same challenges as any high producing cow—the ultimate gains to be captured depend not on the technology *per se*, but on the management skills of its adopters".[19]

### Cloning

The dawn of the era of animal cloning has begun, and has been immediately integrated into the search for means of genetically altering farm animals for hu-

**Table 1.** Endocrine-related biotechnology strategies to enhance bovine growth: alternatives to exogenous bovine somatotropin (bST) administration.[10]

| Strategy | Comments |
|---|---|
| *Regulation of circulating / tissue hormone concentrations* | |
| Administration of growth hormone-releasing hormone (*a.k.a.*, growth hormone-releasing factor [GRF]) | Hypothalamic peptide<br>Stimulates endogenous somatotropin (ST) synthesis and secretion, and has been shown to increase growth and lactation |
| Blocking somatostatin | |
| Produce transgenic cattle overexpressing ST or insulin-like growth factor (IGF-1) | Limited studies available |
| Gene therapy (*in vivo* protein production and delivery) | No information yet in literature<br>Potentially could replace repeated injections with a one-time intervention |
| *Potentiating the potency / physiologic effects of endogenous ST* | |
| Inhibition of corticosteroid activity | *In vitro* data only |
| ST analogs with improved potency | Paradoxically enhance ST biologic effects |
| Antibodies to ST, GRF, somatostatin | |
| Antibodies to somatostatin | |
| *Gene "knock out" technologies* | |
| Myostatin gene | Decreased protein degradation, producing "double-muscled" animal |
| *Cloning* | |
| Genotyping / breeder selection via bioinformatics | Increased risk of susceptibility to disease? Increased emphasis on herd and environmental management |

man benefit. Transgenesis has been defined as the alteration of genomic information with the intent to modify a specific physical trait of an animal.[5] Technologies now exist that allow the creation of genetically hybrid (transgenic) animals, into whose genomes scientists can insert one or more specific genes which have been selectively modified or obtained from other species to favorably modify specific traits in the host animal.

The two primary techniques currently used for the cloning of transgenic animals are microinjection and nuclear transfer. With microinjection, the DNA sequence coding for the desired animal or human protein is introduced directly into a fertilized egg via microinjection. The embryo created in this manner is implanted into a recipient female, which is used as a surrogate mother. With the nuclear transfer method, the gene of interest is introduced into many cultured (and identical) female fetal bovine cells simultaneously. Cells that take up the transgene are identified, isolated, and the population expanded by culturing. The nuclei of these donor cells are then fused to cow oocytes that have been enucleated. The resulting transgenic embryos are implanted into foster mothers and carried to term. Cloned offspring can then produce the gene product (protein) on their own. Nuclear transfer offers the advantage over microinjection of starting with large numbers of identical cultured cells, which minimizes both inefficiencies in the transfer rate as well as genetic variation among founder animals. Incidentally, this was the cloning technique used to generate Dolly the sheep[7] and the first cloned transgenic calves.[8]

## Transgenic Cattle

Obviously, there is interest on the part of the beef industry to identify genes in beef cattle whose proteins influence variation in carcass quality (*e.g.*, muscle tenderness and palatability) and composition (*e.g.*, fat deposition sites and lean tissue yield).[3] The dairy industry is actively applying biotechnologies to the modification of milk content, particularly for the inclusion of human milk proteins that do not exist in unaltered cow's milk.[13] Herman, the first transgenic bull, was born in 1989, carrying the gene for human lactoferrin, an iron-binding protein absent in cow's milk and essential for infant growth. Likewise, animal welfare may benefit from therapeutic interventions that confer resistance to heat, mastitis, bacterial pneumonia, prion diseases such as ovine scrapies and bovine spongiform encephalopathy ("Mad Cow Disease"), and other diseases or environmental factors not easily combated by traditional animal husbandry and breeding techniques.

## "Pharming"—the Production of Recombinant Human Proteins in the Milk of Transgenic Dairy Animals

Perhaps the most familiar family of biotechnologies has been created in the service of producing therapeutic recombinant human proteins in non-human expression systems. Some human proteins can be produced in small quantities via recombinant strategies in non-mammalian expression systems such as bacteria, yeast, and monocots. However, the biological activity of a protein typically depends not only upon its amino acid sequence, but also upon various post-translational modifications specific to mammalian expression systems (*e.g.*, refolding and glycosylation). However, the cost to maintain mammalian cells in culture is high relative to the small amounts of recombinant protein produced. Therefore, there is tremendous need to develop alternative and economically feasible mammalian expression systems capable of creating large amounts of therapeutically relevant proteins. This is particularly true for protein therapeutics that are required on a massive scale, such as human blood products (*e.g.*, clotting factors and serum albumin) and antibodies. The high cost of antibody production using current methodologies can negate their therapeutic utility in chronic diseases such as cancer, where repeated administration of large doses of highly purified recombinant antibody may be required.[17]

Recently, a great deal of attention has been focused upon the development of transgenic bioreactor animals for "gene pharming" (***ph***armaceutical f***arming***). The basis for this concept is that transgenic dairy animals can be genetically engineered to express a human recombinant protein of therapeutic utility. The expression of the recombinant protein can be targeted to the mammary gland, where large quantities (often between 1 and 10 grams per liter) can be easily harvested using traditional dairy and protein isolation techniques. DNA coding for the desired human protein is spliced to DNA coding for the promoter of an endogenous milk protein, such as $\alpha_{s1}$-casein, forming a hybrid gene (transgene).[21] The transgene is either introduced directly into fertilized eggs (microinjection) or cultured in cells whose nuclei are then introduced into enucleated eggs (nuclear transfer). The eggs are then implanted in recipient female animals. By virtue of the presence of the milk protein promoter sequence, expression of the resulting hybrid (chimeric) protein is directed to the mammary gland and regulated by the normal lactation control mechanisms in the host animal. Transgenic founder animals (genetic founders) transmit the transgene to their offspring (production founders) in Mendelian fashion, and the production herd is selected on the basis of recombinant protein production data obtained from the collected milk.

This strategy has been successfully implemented in various transgenic farm animal systems, including bovine (Table 2). Transgenic cattle are preferred when the amount of protein needed is sufficiently great to outpace the desire to have the shorter production herd timelines afforded by smaller species such as mice, rats, rabbits or goats. Milk is initially obtainable from a transgenic cow about 6 months after birth.[6]

The potential economic benefits of this technology are startling. Transgenic cows can inexpensively produce large volumes of milk (up to 10,000 L / yr) containing only biologically safe material,[23] from which the desired therapeutic protein can be easily harvested (up to 220 lb (100 kg) yield / yr). With such a rate of transgenic protein production, transgenic cattle herds consisting of 1-17 animals each have been predicted to meet the total annual need for human blood coagulation proteins Factor VIII, Factor IX and fibrinogen, while a herd of 35,000 would be required to meet the annual demand for human serum albumin.[22] Scale-up is flexible, since the size of an existing herd can be readily adjusted to match demand.

As with traditional cattle herd management, conventional breeding techniques can be supplemented with newer methodologies to maximize bovine production efficiency. The establishment of a production herd of sufficient size could be enhanced via assisted reproduction technologies such as superovulation of pedigree animals, artificial insemination, *in vitro* embryo culture and manipulation, and embryo splitting and transfer. *In vitro* progeny testing will automatically increase the efficiency of transgenic production herd generation by 50% since with single chromosome insertion only half of the offspring will inherit the transgene. Prepubertal

**Table 2.** Partial list of human proteins in development for directed expression to the mammary gland of transgenic cows.[6,14]

| Protein | Function and/ or disease indication | Developmental stage | Company/site |
|---|---|---|---|
| Serum albumin | Hemorrhage (surgery adjunct for blood volume expansion); hormone transport | Preclinical | Genzyme Transgenics & Fresenius |
| Myelin basic | Multiple sclerosis | Preclinical | (New Zealand) |
| Lactoferrin | Iron supplement for milk (not present in cows' milk); antibiotic, probiotic and anti-inflammatory properties; in clinical trials for heparin neutralization and arthritis | √ expressed; Phase I clinical trials (for both indications) | Pharming NV (Netherlands) |
| Collagen type I | Structural protein (tissue repair, wound healing) | Preclinical | Pharming, Cohesion Technologies / Collagen Aesthetics |
| Collagen type II | Structural protein (rheumatoid arthritis) | Preclinical | |
| Fibrinogen | Blood clotting factor (hemostatic) | Preclinical | Pharming & American Red Cross |
| Factor VIII | Blood clotting factor absent in Hemophilia A (hemostatic) | Preclinical | Pharming & American Red Cross |

reproduction is now possible, via oocyte retrieval as early as 2-4 months followed by *in vitro* fertilization and transfer to surrogate mothers. While normal lactation typically does not commence before two years of age, hormonal induction of lactation can be used at 2-6 months of age.[16]

Recombinant proteins produced in this manner are typically harvested from the milk whey fraction and removed using chromatographic procedures commonly used in the dairy industry. The nature of the specific protein produced and its anticipated use determine the subsequent purification procedure used. For most therapeutic proteins, rigorous purification is required, as determined by Good Manufacturing Practices specifications.

There remain significant challenges to the widespread adoption of gene pharming as a standard biotherapeutic production methodology. Cattle have proven notoriously difficult for transgenic generation by comparison to other species.[11] There remains a need to improve procedural efficiencies. Microinjection-based techniques for transgenic cattle currently demonstrate a 5-20% rate of success and require 78 months to produce a herd of lactating transgenic cows.[6] Inherent variability exists among donor nuclei and recipient cytoplasm. Presumably, each of these issues can be overcome by the adoption of nuclear transfer as the preferred cloning technology, since it can be used to produce large numbers of identical, transgenic bovine embryos simultaneously, reducing the time to herd generation to 33 months,[6] and further refinement of genetic engineering approaches are likely to be forthcoming with the recent generation of embryonic stem-like cells.[9,18] Relatively low pregnancy rates are problematic, as are health problems observed in cloned offspring.[12] However, the rapid technological advances experienced so far in this very young area suggest optimism for the resolution of these issues.

## Regulatory Responsibilities

Several USDA agencies are involved in regulating and monitoring the use of biotechnology for agriculture. The following is quoted from the USDA Biotechnology Website (http://www.aphis.usda.gov/biotech/usda_biotech.html):

The Animal and Plant Health Inspection Service (APHIS) regulates the movement, importation, and field testing of Genetically Engineered Organisms (GEOs) through permitting and notification procedures. In addition, APHIS Veterinary Biologics inspects biologics production establishments and licenses genetically engineered products.

The Food Safety Inspection Service (FSIS) has responsibility for the safe use of engineered domestic livestock, poultry, and products derived from them.

The Foreign Agricultural Service (FAS) monitors foreign regulations and restrictions of Genetically Engineered Organisms.

The Agricultural Research Service (ARS) conducts in-house research on GEOs and the Economic Research Service (ERS) conducts research on the economic impact of GEOs. The Cooperative State Research, Education, and Extension Service (CSREES) funds the biotechnology risk assessment program, and research in gene mapping, sequencing and biotechnology applications.

The Agricultural Marketing Service (AMS) will be responsible for administration of new organic labeling requirements.

## Challenges

Other significant challenges exist relative to the acceptance of the incorporation of various biotechnologies into the beef and dairy cattle industries.[15] Governmental policies regarding the use of biotechnologies relating to food animals are not fully developed and vary across countries. Worries regarding the safety of genetically modified foods (whether of plant or animal origin) have little scientific substantiation. Nonetheless, feelings of concern on the part of the general public have been neither entirely validated nor alleviated by the biotechnology or food industries. It is likely that at least some of the burden of proof regarding the innocuous nature of "value-added" foods will fall to the food industries.

Public concern for animal welfare previously prompted the adoption of standards of care for animal use in research, which forced scientists and their associated institutions to demonstrate humane treatment of research animals and the adoption of study designs that minimize the numbers of animals required. It is likely that the public will be no less adamant that tangible assurances are made regarding the conscientious treatment of animals used for biotechnology-assisted food or therapeutic production. Frequently, scientific considerations parallel the associated ethical issues. For example, erythropoietin (a hormone which signals for the production of red blood cells) is not produced in transgenic mice because small amounts get into the bloodstream, causing the overproduction of RBC and subsequent death of the animals.

The other ethical dilemma concerns the generation of genetically modified organisms of any kind. While the engineering of transgenic yeast and bacteria has not been perceived by the public as a social threat or philosophic issue, the same cannot be said for genetically modified higher organisms. Public opinion is currently sharply divided, and discussions of these issues are easily found elsewhere.

Questions of societal acceptance of a role for biotechnology in the beef and dairy industries extend to the farmers themselves. For example, despite having been available commercially for over 40 years, artificial insemination technology is used only by 65 to 70 percent of dairy farmers. Likewise, Dairy Herd Improvement (DHI) technology, available for 50 years, is used by only 45 percent of farmers.[20] The use of both technologies has been characterized by regional variation. In addition, the economic question arises as to whether the patenting of transgenic animals and their subsequent licensure or sale may be cost prohibitive to the farmer with a small herd.

In any event, the bovine practitioner, the consumer and the government will each be forced to balance perceived risk with perceived reward. "The rate-limiting event that will determine the commercial and societal benefits of the emerging biotechnologies will not be the scientific discoveries made, as impressive as they are (and will be), but the extent to which the public perceives the benefits and embraces the need to support the commercial development and adoption of these new products."[10]

## Acknowledgements

The authors wish to acknowledge the helpful assistance of Nancy Kozlowski (Library and Information Services), Barry Teater, and Dr. Ken Tindall of the North Carolina Biotechnology Center.

## References

1. Band MR, Larson JH, Rebeiz M., Green CA, *et al.*: An ordered comparative map of the cattle and human genomes. *Genome Research* 10: 1359-1368, 2000.
2. Bauman, DE: "Bovine Somatotropin: Review of an Emerging Animal Technology," Commissioned background paper for the U.S. Congress Office of Technology Assessment, Washington. DC, 1990.
3. *Beef Cattle Genomics: Past, Present and Future*, from the Beef Genomics Workshop proceedings (College Station, TX; Oct. 15-17, 1997), http://www.beef.org/library/publications/research/genomcov.htm
4. Bonneau M, Laarveld B: Biotechnology in animal nutrition, physiology and health. *Livestock Production Sci* 59: 223-241, 1999.
5. Brink MF, Bishop MD, Pieper FR: Developing efficient strategies for the generation of transgenic cattle which produce biopharmaceuticals in milk. *Theriogenology* 53: 139-148, 2000.
6. Burke M: Farmyard tales: milking transgenic animals for human proteins. *Bioventure View,* Dec. 1999.
7. Campbell KH, McWhir J, Ritchie WA, Wilmut I: Sheep cloned by nuclear transfer from a cultured cell line. *Nature* 380: 64-66, 1996.
8. Cibelli JB, Stice SL, Golueke PJ, Kane JJ, *et al.*: Cloned transgenic calves produced from non-quiescent fetal fibroblasts. *Science* 280: 1256, 1998.
9. Cibelli JB, Stice SL, Golueke PJ, Kane JJ, *et al.*: Transgenic bovine chimeric offspring produced from somatic cell-derived stem-like cells. *Nature Biotech* 16: 642-646, 1998.

10. Etherton TD: Emerging strategies for enhancing growth: is there a biotechnology better than somatotropin? *Domestic Animal Endocrinol* 17: 171-179, 1999.
11. Eyestone WH: Production of transgenic cattle expressing a recombinant protein in milk, In *Transgenic Animals in Agriculture*. JD Murray, GB Anderson, AM Oberbauer, MM McGloughlin, *eds*, CABI Publishing, New York, NY, 1999.
12. Hill JR, Rousell AJ, Cibelli JB, Edwards JF, *et al.*: Clinical and pathologic features of cloned transgenic calves and fetuses (13 case studies). *Theriogenology* 51: 1451, 1991.
13. Karatzas CN, Turner JD: Toward altering milk composition by genetic manipulation: current status and challenges. *J Dairy Sci* 80: 2225-2232, 1997.
14. Krimpenfort P, Rademakers A, Eyestone W, van der Schans A, *et al.*: Generation of transgenic cattle using 'in vitro' embryo production. *Bio / Technology* 9:844-847, 1991.
15. Mench JA: Ethics, animal welfare and transgenic farm animals, In *Transgenic Animals in Agriculture*. JD Murray, GB Anderson, AM Oberbauer, MM McGloughlin, *eds*, CABI Publishing, New York, NY, 1999.
16. Narendran R, Hacker RR, Smith VG, Lun A: Hormonal induction of lactation: estrogen and progesterone in milk. *J Dairy Sci* 62: 1069-1075, 1979.
17. Pollock DP, Kutzko JP, Birck-Wilson E, Williams JL, *et al.*: Transgenic milk as a method for the production of recombinant antibodies. *J Immunol Methods* 231: 147-157, 1999.
18. Stice SL, Gibbons J, Rzucidlo SJ, Baile CA: Improvements in nuclear transfer procedures will increase commercial utilization of animal cloning – review. *Asian-Aus J Animal Sci* 13: 856-860, 2000.
19. U.S. Congress, Office of Technology Assessment, *U.S. Dairy Industry at a Crossroad: Biotechnology and Policy Choices – Special Report* (May 1991) OTA-F-470 (Washington, D.C.: U.S. Government Printing Office). http://www.nal.usda.gov/bic/BST/ndd/US_DAIRY_INDUST_AT_A_CROSSROAD_PART_1_OF_5.html
20. U.S. Department of Agriculture. National Economic Indicator Farm Cost and Return Survey. Washington, DC: Economic Research Service, 1985.
21. Wall RJ, Kerr DE, Bondioli DR: Transgenic dairy cattle: genetic engineering on a large scale. *J Dairy Sci* 80: 2213-2224, 1997.
22. Wall RJ: Biotechnology for the production of modified and innovative animal products: transgenic livestock bioreactors. *Livestock Production Sci* 59: 243-255, 1999.
23. Wright G, Carver A, Cottom D, Reeves D, *et al.*: High level expression of active human a-1 antitrypsin in the milk of transgenic sheep. *Bio / Technology* 9: 77-84, 1991.



# Temporal aspects of the epidemic of bovine spongiform encephalopathy in Great Britain: holding-associated risk factors for the disease

J. W. Wilesmith, J. B. M. Ryan, M. A. Stevenson, R. S. Morris, D. U. Pfeiffer, D. Lin, R. Jackson, R. L. Sanson
*Veterinary Record* (2000) 147, 319-325

The objectives of this study were first to describe the pattern of the epidemic of bovine spongiform encephalopathy (BSE) in Great Britain in terms of the temporal change in the proportion of all cattle holdings that had experienced at least one confirmed case of BSE to June 30, 1997, and secondly to identify risk factors that influenced the date of onset of a holding's first confirmed BSE case. The analyses were based on the population of British cattle at risk, derived from agricultural census data collected between 1986 and 1996, and the BSE case data collected up to June 30, 1997. The unit of interest was the cattle holding and included all those recorded at least once on annual agricultural censuses conducted between June 30, 1986, and June 30, 1996. The outcome of interest was the date on which clinical signs were recorded in a holding's first confirmed case of BSE, termed the BSE onset date. Univariate and multivariate survival analysis techniques were used to describe the temporal pattern of the epidemic. The BSE epidemic in Great Britain started in November 1986, with the majority of affected holdings having their BSE onset date after February 1992. After adjusting for the effect of the size and type of holding, holdings in the south of England (specifically those in the Eastern, South east and South west regions) had 2·22 to 2·43 (95 per cent confidence interval [CI] 2·07 to 2·58) times as great a monthly hazard of having a BSE index case as holdings in Scotland. After adjusting for the effect of region and type of holding, holdings with more than 53 adult cattle had 5·91 (95 per cent CI 5·62 to 6·21) times as great a monthly hazard of having a BSE index case as holdings with seven to 21 adult cattle. Dairy holdings had 3·06 (95 per cent CI 2·96 to 3·16) times as great a monthly hazard of having a BSE index case as beef suckler holdings. These analyses show that there were different rates of onset in different regions and in holdings of different sizes and types, that the epidemic was propagated most strongly in the south of the country, and that the growth of the epidemic followed essentially the same pattern in each region of the country, with modest temporal lags between them. The control measures imposed in 1988 and 1990 brought the expansion of the epidemic under control, although the rate of progress was slowed by those regions where the effectiveness of the control methods took some time to take full effect.