

FAO/IAEA International Symposium on
*Applications of Gene-based Technologies for Improving Animal
Production and Health in Developing Countries*

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**JOINT FAO/IAEA DIVISION
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Executive summary

Research aimed at improving animal production and health has been revolutionized by recent developments in biotechnology, particularly those involving gene-based technologies. These have generated not only new opportunities for knowledge creation but also new options for solving established and emerging problems.

The underlying theme of this Symposium was the exploitation of gene-based technologies for use in the livestock sector in developing countries. In this regard, consideration was also given to inherent problems in developing countries, given their weak research base and lack of training, which in turn limits their ability to effectively utilize gene-based methods. The contrast between the resources available to developing and developed countries were clearly evident. However, even in technologically advanced countries, there are relatively few examples of novel biotechnological products that can demonstrate key advantages to people and communities. That is not to say that the potential of gene-based technologies were understated, only that tangible proof of practical use was lacking. The main problem seemed to be one of ensuring large-scale, sustained funding of such technologies in the livestock arena. It did, however, become clear that less-developed countries might benefit even more than developed countries from advances in genetic research. Nevertheless, a concerted effort is needed to understand real needs and to make adjustments for the longer developmental time scales needed. It is clear that genetic analyses are already well established, or at least readily achievable in most developing countries, although genomic analysis still rests with a few specialized centres in the developed world.

The scientific information presented at this symposium was of a nature that elicited much excitement regarding the potential applications for gene-based technologies, particularly in those field that have matured more, notably in animal disease diagnostics and therapeutics, rather than in animal production. Developing countries could thus acquire more economic benefits in the short term by focusing research and development work on animal health. In the area of animal production, benefits are more likely to accrue in the medium to long term. The timing of the Symposium was opportune in the face of various recent disease epidemics and the continuing debate on the “livestock revolution” that is expected to address global food requirements and influence trading patterns.

Animal Production

1. The genetic manipulation of grain crops and tropical forages to increase the efficiency of nutrient availability to animals and to decrease phosphorus and nitrogen emission into the environment offers considerable potential in increasing livestock productivity while protecting the environment. This could be achieved through molecular manipulation, transgenesis, and site-directed or conventional mutation breeding.
2. International and national bodies should further promote the use of quantitative methods for polymerase chain reaction (PCR) and oligonucleotide probe-based analysis for reducing methane, countering the effects of antinutritional factors, monitoring gut pathogens associated with food safety, improving utilization of feed resources, and in identifying new pro- and pre-biotics and antibiotic substitutes for addition to feeds in developing countries.
3. The practical applications of genomic studies on rumen microbes are currently unpredictable, but could involve industrial production of key lignocellulytic enzymes for

pre-treatment of fibrous residues. Rumen genomic studies might provide insight into the nutrient requirements of these organisms and this knowledge could be employed in practical feeding strategies. Because of present uncertainties and the high cost of genomic research, developing countries should preferably not initiate such studies, but should rather direct resources to enhancing capacity in the area of bio-informatics, since sequence information will become publicly available.

4. In developing countries, the use of genotype information is likely to be more useful in marker-assisted introgression than in selection within breeds. Introgression of a single mutated gene is relatively easy and holds promise for livestock improvement in developing countries and should thus be targeted. Some single-gene mutations are known and information on many more is likely to become available in the near future. A model for introgression is the Booroola gene of the Garole breed, as demonstrated in India and which could be replicated in other countries.
5. Locally adapted breeds should be characterized for nutritional resilience, behaviour, metabolic rates, energy expenditure, muscle and bone structure, feed conversion efficiencies, female reproductive performance and lactation, protein turnover, mobilization of energy for lactation, etc. In low-input systems in developing countries, complete phenotypic and pedigree information is often not available except in some intensive breeding units. The real value of marker information will thus be under-utilized. A proper recording system must also be in place if the full potential of marker-assisted selection for breed improvement is to be realized.
6. If transgenic animals were to gain acceptance in developing countries, then gene constructs and transgenic technology should be used to modify locally adapted breeds rather than introducing elite transgenic breeds from developed countries. It should be noted that transgenesis is unlikely to be successful for genes encoding traits governed by gene networks, although it is feasible, and should be pursued in the case of single-gene traits.

Animal Health

1. The increasing demand for and trade in animals and animal products is fuelling a livestock revolution in developing countries and posing increased health problems, to both animals and man. Gene-based technologies are playing an increasingly important role in addressing such problems.
2. Major advances have been made in elucidating host-pathogen interactions, diagnosis, molecular epidemiology and predictive epidemiology of veterinary diseases, as well as in vaccine production, through the exploitation of gene-based technologies. Major progress has also been made in the sequencing of animal genomes.
3. Different solutions are required to solve problems of animal health and food safety in developed versus less-developed countries. For the latter, more appropriate exploitation of gene-based technologies could be identified. Developed countries tend to regard developing countries as sources of disease and a threat. There is also a large differential in the attitudes towards various diseases according to the state of economic development. The prospect for increased trade is a major factor in promoting gene-based methods and research in developing countries. More appropriate targeting of research by developed

countries is needed to help produce more relevant vaccines and diagnostic methods for transfer to developing countries.

4. Training is an essential pre-requisite to allow pure and applied research, and, by extension, to produce appropriate reagents, therapeutics and vaccines to deal with problems peculiar to developing countries.
5. Molecular tools such as PCR and micro-arrays have driven developments, particularly within the fields of disease diagnosis and control. Molecular epidemiology based on sequence comparisons are now commonplace, as also real-time and forensic analyses of diseases and pathogens.
6. Gene-based technologies allow for more direct, non-invasive diagnostic methodologies within a short time frame. An increasing number of faster and more sophisticated molecular diagnostic techniques based on PCR are available for the detection of cellular and viral pathogens. Sensitive and specific techniques will be more readily usable at the site of infection, but the challenge remains of ensuring validation to ascertain true diagnostic sensitivities. Care is needed to ensure that existing serological tests remain in use in tandem with molecular methods.
7. Gene-based technologies have already made a major impact on animal vaccinology. As an illustration, widespread studies are in progress on the use of naked DNA vaccination. For control programmes, it is important that newly designed vaccines include markers to distinguish vaccinated from infected animals. The concept was first tested using DNA viruses whose genomes could easily be manipulated using molecular techniques. Reverse genetics of viral pathogens can provide new immunogenic constructs that act as marker vaccines for many RNA viruses. The development of vaccines against invertebrate disease vectors so as to directly attack the source of the transmission and spread of many important human and veterinary pathogens, offers exciting possibilities. Major constraints on vaccine development occur where there is a high rate of mutation and selection of a pathogens, requiring the availability of multivalent vaccines. The physical and environmental stability of such vaccines must also be assured.

Education, intellectual property rights, ethics, biosafety and related aspects

1. Technologies and expertise should be transferred to developing countries through capacity building programmes whose aims and focus are driven by the countries themselves. Appropriate modalities for achieving this must be determined. Equally important is institutional strengthening for strategic planning, priority setting and policy development for identification of appropriate gene-based technologies and their applications. Regional and subregional approaches should be used to enhance cooperation, capacity building and sharing of knowledge.
2. Establishing and strengthening the networking of groups in developing countries for research and development work in 'modern biotechnology' and application of gene-based technologies will provide the necessary impetus to address the emerging challenges for enhancing animal productivity and health. Increased contact between academics and industry is essential, and is one of the main prerequisites to becoming more competitive in biotechnology. Initiatives to stimulate such interactions should be encouraged and actively supported.

3. There is an urgent need to involve industry in the commercialization of research products, especially in the early stages where venture capital plays an important part in the developed world.
4. Intellectual property rights (IPR) and the use of patented products and procedures are important issues to consider with any application of genomic-based procedures worldwide, including in developing countries. There is a need for the international research community to coordinate their activities efficiently in order to address issues of IPR – issues that have often limited collaboration among public and private institutions.
5. Issues of ethics and biosafety need to be addressed at the institutional and national levels, but harmonization should be at inter-governmental or international level.
6. Gene-based technologies are unlikely to have an impact if applied in isolation. Programmes that work to understand and improve the full socio-economic system of animal production and marketing are more likely to succeed than those that deal with individual components of the system.
7. International funding agencies should assist and support countries to ensure that gene-based technologies are deployed within the framework of national development aspirations.

FAO/IAEA International Symposium on *Application of Gene-Based Technologies for Improving Animal Production and Health in Developing Countries*

Summary, Conclusions and Recommendations

The symposium was held from 6 to 10 October 2003 in Vienna. One hundred and thirty scientists and decision-makers from 60 Member States participated in the Symposium. A total of 44 oral and 33 poster presentations were made. The programme consisted of opening addresses, an opening session to set the scene and four scientific sessions covering, respectively, animal breeding and genetics; animal health; animal nutrition; and environmental, ethical, safety and regulatory aspects of gene-based technologies. There were also three panel discussions. In the opening address session, three distinguished speakers (Werner Burkart, DDG and Head of the Department of Nuclear Sciences and Applications, IAEA; Samuel Jutzi, Director, Animal Production and Health Division, FAO; and James Dargie, Director, FAO/IAEA Division of Nuclear Applications in Food and Agriculture) presented their views. Mr Burkart stressed the importance of the close relationship between FAO and IAEA for enabling the exploitation and deployment of nuclear technologies in food and agriculture. Mr Jutzi stressed the challenges and opportunities faced by animal agriculture globally, and emphasized the importance and nature of specific and general development policy measures for enhancing the impact of gene-based technologies in animal agriculture in developing countries. Mr Dargie emphasized the need for training, technical support and capacity building in developing countries for enabling the application of gene-based technologies in key areas of the livestock sector.

Opening session: Setting the scene for the international symposium

Samuel Jutzi

Director, Animal Production and Health Division, FAO, Rome

Two keynote addresses were presented: *A vision of gene-based technologies for the livestock industries in the third millennium* by E.P. Cunningham, and *Challenges and opportunities for controlling and preventing animal diseases in developing countries through gene-based technologies* by M.H. Jeggo and J.R. Crowther. The salient points that emerged from this session are summarized below.

On the technological front

1. The vast expansion of knowledge in the last ten years in the development of new techniques for isolating, amplifying, reading and inserting DNA, the up-scaling and automation of those techniques, and their coupling with information management suggests the continued and accelerated achievement of breakthroughs in the exploration and management of gene function.
2. One of the largest impacts of gene-based technologies on livestock sector so far, given the large economic significance of feed in livestock production, has been through their

application in maize and soybean transformation (reduced production costs, reduced mycotoxin levels, higher P- and N- digestion efficiencies, better balance of amino acids).

3. Non-contentious and fast advancing gene-based technology frontiers are those that relate to genome mapping, exploration of genetic diversity, DNA typing, fingerprinting, genetic tracking, marker technology and functional genomics. Transgenesis is of little concern as long as it is only used for vaccine generation. The need to further strengthen efforts in the production of more efficient vaccines was, however, stressed.
4. Public concerns about animal transgenesis for purposes related to growth and nutritional performance, health management and pharmaceuticals or organ supply for human medicine, as well as the application of genetically modified growth hormones, has tended to slow both technological progress and the market introduction of such products and processes.

In the functional context

1. Gene-based biotechnology requires an appropriate policy and institutional framework to exploit its full potential. Emphasis should be on developing relevant and effective livestock sector policies, and on development of institutions, organizations and services.

Session I: Gene-based technologies applied to livestock genetics and breeding

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Four keynote addresses were presented. These focused on (1) approaches, opportunities and risks of molecular genetics and livestock selection; (2) technologies for germline manipulation; (3) state of the world's animal genetic resources; and (4) integration of gene-based methods and reproductive technologies for genetic improvement of livestock. Molecular genetic and genomic technologies have been applied to gene detection, genetic selection and assessment of genetic diversity and genetic transformation of livestock for the past 20 years. Research has taken place mostly in the developed world and these technologies and their applications are still a young science, but consideration must be given to adapt these technologies to meet the specific needs of developing countries. The paper on the state of world's animal genetic resources revealed a wide gap between developed and developing world in research, development and applications of gene-based technologies. Developing countries should be prepared to receive and fully exploit these technologies.

1. Genetic improvement of livestock is already very effective using conventional technologies, and new technologies, such as use of molecular genetic markers, must compete with these conventional approaches. Molecular genetic markers are recognized as being useful and cost-effective technologies that can be incorporated into genetic improvement programmes to improve efficiency or rate of genetic gain, or both, but they do not in themselves provide large extra genetic gains and nor do they substitute for existing methods of genetic improvement. There are opportunities for application in the developing world, but the difficulties in terms of needs for advanced training and development of technical capacity should not be underestimated.
2. Marker assisted selection is mainly useful for traits where phenotypic measurement is less valuable because of low heritability, sex-limited expression, non-availability before sexual maturity, or unavailable without sacrificing the animal (e.g. slaughter traits). Traits such as feed intake or disease resistance may also be expensive or difficult to measure, and information on genotype might be useful in selecting for these traits. Genotypic information has extra value in the case of early selection and where within-family

variance can be exploited. Reproductive technologies usually lead to early selection and more emphasis on between-family selection. DNA marker and reproductive technologies are therefore highly synergistic and complementary. In the low-input systems existing in developing countries, complete phenotypic and pedigree information is often not available, except in some intensive breeding units. Under these scenarios it would be more difficult to realize the value of the marker information, and it would be harder and more expensive to determine linkage in the case of using linked markers. A proper recording system must be put in place if the full potential of gene-based technologies for breed improvement is to be realized.

3. There are opportunities for application of molecular genetic approaches in the developing world. Rather than exploiting existing quantitative trait loci (QTL) through within-breed selection, a more likely scenario for developing countries will be that valuable QTL will be introgressed from one population into another. In developing countries there is a huge variation across breeds, much of it being useful to exploit in genetic improvement programmes. This includes the variation coming from 'foreign' breeds in developing countries. Indigenous breeds may contain valuable QTL, but could benefit from upgrading through crossing with superior exotic breeds. Alternatively, valuable QTL could be introgressed from exotic breeds. Examples are the Booroola gene in the Garole breed in India, having a moderate and desirable effect on number of lambs weaned, and a number of genes affecting resistance to endemic local diseases. There are many cases of QTL found in crosses of extreme breeds, and a number of those will be candidates for introgression. In developing countries, use of genotype information is therefore probably going to be more useful in marker-assisted introgression (MAI) rather than in selection within breeds. Also, in the case of MAI, reproductive technologies will be beneficial because they can help increase the number of animals with the desired genotype.
4. Information is currently available for a number of 'direct markers' such as myostatin affecting double muscling in cattle, calipyge doing the same in sheep, and Booroola affecting fecundity. Many of these mutations have a major phenotypic effect. Even if the genetic marker were a direct marker, its effect on phenotype would have to be estimated for the population and the environment it is used in. The effects of individual genes are likely to be dependent upon the background, which must also be characterized. Phenotypic information may be less used in actual selection in the future, but recording of phenotype will be continuously needed for the purpose of monitoring the QTL effect (retrospectively), and genetic change over time.
5. Genetically modified (GM, transgenic) livestock have not yet entered commercial production. The technology of producing GM livestock remains expensive and difficult, but recent breakthroughs promise substantial reductions in cost and increases in efficiency in the near future. Similarly, increasing knowledge of mammalian and avian genetics and genome function is opening new avenues that could be of economic and social value in both developed and developing countries. Opportunities to create disease resistance seem particularly promising, but applications for livestock improvement in developing countries are likely to be medium to long term rather than short term.
6. The ongoing FAO survey of the State of the World's Animal Genetic Resources has already revealed several consistent demands from developing countries for biotechnology applications. Demands for breed characterization, for cryopreservation of livestock germplasm and for application of embryo transfer technologies were highlighted. There

was little expressed demand for advanced genetic technologies, and the gap between the developed and developing worlds in such technologies was clear. Where demand for advanced biotechnologies was expressed, there was little evidence of an underlying strategic plan. These results identify needs for capacity development and technology access in advanced biotechnologies and strategic planning.

7. The country reports within the State of the World's Animal Genetic Resources also confirm observations that changes in status and risks to indigenous livestock genetic resources in West Africa are very rapid, and that several breeds are already extinct and others are under severe threat. Interventions will therefore need to be rapid and comprehensive to prevent permanent loss of a substantial proportion of existing genetic resources. Global surveys and characterization of livestock diversity, within which molecular diversity is a component, should be completed as an essential starting point for informed actions on conservation and utilization. International support should be provided to accomplish this. An international panel should also be established to review and recommend procedures for characterization and conservation of animal genetic resources. The question of when and how *in vitro* conservation techniques should be applied needs to be defined and specific recommendations provided to users.
8. Gene-based technologies and genetic improvement in general are unlikely to have any impact if applied in isolation. Rather, it is critically important to work with local communities to identify what are the real needs and opportunities for achieving a positive impact on people's lives, and then to work with those communities to test and apply improvements. Programmes that work to understand and improve the full socio-economic system of animal production and marketing are more likely to succeed than programmes that are limited to individual components of the system.

Session II: Gene-based technologies applied to pathogens and host-pathogen interactions

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Gene-based technologies applied to animal health must fall in line with the general trends and the needs of animal production and health in developing countries. As indicated in the talk given by Samuel Jutzi, an increasing demand for animal products is foreseen in developing countries over the next 20 years – the so-called “livestock revolution”. This increased demand will no doubt be accompanied by an increase in the exchange of animals and animal products, with consequent greater risks of transboundary spread of disease. There are therefore likely to be major problems concerning food security in developing countries, not forgetting the many problems associated with food safety.

As exemplified by the recent outbreaks of foot-and-mouth disease and classical swine fever in Europe, the control of OIE List A diseases in developed countries is still achieved through the implementation of sanitary policies, which include mass slaughtering of infected and in-contact animals. One cannot expect these measures to be adopted in developing countries where food security has not yet been achieved. In any event, public opinion in developed countries is increasingly concerned with animal welfare and the ethical justification of a mass slaughter policy; this has led to the new concept of “vaccination for life”. Additional

biological facts relating to developing countries must be taken into account, such as the high level of animal biodiversity in some of them and the existence of wildlife reservoirs of infections and infestations, which can be a source of new emerging diseases in the human and domestic animal populations. Compared with wildlife species, the existence of domestic livestock is a recent event in evolutionary terms, and therefore the domestic animal populations have had little time to adapt to certain pathogens. Coupled with this is the fact that the evolutionary rate of viruses is of another order of magnitude compared with that of their host species, and leads to the establishment of quasi-species populations with multitudes of variant genomes. To date, a total of about 3 600 viral species and their variants have been described.

Animal health is an important component of sustainable agriculture; however, biosecurity – the need to protect the environment and maintain biodiversity – must also be taken into account. By the end of the last century, molecular biology had made outstanding progress. Molecular tools – PCR, micro-array technology, etc. – had all made a substantial impact in the field of disease diagnosis and control. The complete genome sequences of the majority of relevant viruses, as well as those of numerous bacteria, are now available, and the completion of the human genome sequence has been achieved. Genome sequence information will soon be available for most of the important livestock species, including poultry, pigs and cattle. These advances will also affect the use of gene-based technologies in animal health in developing countries, and this will need to be taken into account in future studies on host–pathogen interactions (interactome).

Impact of gene-based technologies on animal health

The impact of gene-based technologies on animal health has primarily been on infectious or parasitic diseases, and these have made important contributions in four main areas:

- host–pathogen interactions;
- diagnosis;
- molecular epidemiology and predictive epidemiology; and
- vaccines.

Nowadays, the three disciplines of epidemiology, diagnosis and vaccinology are more closely linked than ever, since one of the main advances in veterinary vaccinology has been the development of marker vaccines, obtained by recombinant DNA technology. These marker vaccines, used for “vaccination for life”, must, however, be linked with suitable companion diagnostic tests. Epidemiological studies will then indicate if vaccination campaigns are necessary and, if so, whether or not they are successful.

It is worth keeping in mind that these three disciplines, when translated into practice, have no negative impact on animal welfare, animal rights or animal integrity; in fact, the use of vaccines should improve animal welfare.

1. Host-pathogen interactions

New gene-based technologies such as micro-arrays and knowledge of genomic sequences of both the pathogen and the host have allowed more refined pathogenesis studies to be carried out, i.e. the “interactome” between gene products of the pathogen and those of the host.

It is becoming obvious that many viruses, microbes and parasites are very “clever” at evasion, they have co-evolved with their hosts and therefore it can sometimes be very difficult to find the best way to fight them. This brings to mind a well-known paper by Rolf

Zinkemagel entitled *Immunology taught by viruses* and no doubt they will also “teach” us much about pathogenesis.

2. *Diagnosis*

Serological, indirect methods of diagnosis of infectious diseases are still very useful but have some drawbacks, such as the lag period between the initial infection and the appearance of detectable amounts of antibodies. Gene-based technologies are allowing us to increasingly use more direct non-invasive diagnostic methodologies in a more rapid time frame.

There are an increasing number of faster and more sophisticated molecular diagnostic techniques based on PCR available for the detection of cellular and viral pathogens. These are suited to surveillance of pathogens in environmental samples (water, soil, etc.), in food products and in biological specimens from humans, animals and plants. Nucleic acid fingerprinting and sequencing can be applied to the identification of pathogens (species, types, subtypes, variants, etc.). Portable instruments enabling this work to be carried out in the field are also becoming available.

For diagnostic applications in developing countries, the main recommendations are to:

- adopt technologies that use fluorescent probes and accessible bio-informatic programmes;
- develop diagnostic tools that can be used in the field (pen-side technologies);
- train personnel in the techniques to ensure proper interpretation of positive and negative results; and
- establish networks of epidemio-surveillance laboratories that function in an integrated manner.

3. *Epidemiology*

Molecular epidemiology is one of the most powerful applications of gene-based technology in animal health. Bearing in mind that viruses, especially RNA viruses, are quasi-species, that is to say highly variable, it is very important to be able to rapidly identify and characterize them to enable effective control measures to be implemented without undue delay. A further development could be the new science of predictive epidemiology using sequence data, and also micro-arrays, to search for new pathogens. As exemplified by several recent examples (Hendra, Nipah, SARS, etc.), wildlife can act as reservoirs for numerous potentially emerging, often zoonotic, infections. It would now be feasible to check for potential disease risks in wildlife using highly conserved regions of nucleic acid sequence in known viral genomes.

For application to epidemiological surveillance in developing countries, two main recommendations emerge:

- It is essential to implement surveillance of the genotypic or antigenic types and variant forms of the pathogens to be controlled (see also under *Diagnosis*). Phylogenetic methods to identify lineages and to trace the origin of pathogens and quantitative relatedness among different forms of the same pathogen should be used systematically, notably for predictive epidemiological purposes.
- Studies on wildlife should be undertaken, especially in regions with very high animal biodiversity

4. *Vaccinology*

Compared with other technologies, vaccines offer the single most cost-effective measure to control or even eradicate an infectious disease, as exemplified by the eradication of smallpox, the near eradication of rinderpest and the foreseen elimination of wildlife rabies from

mainland Europe. There are no broad spectrum antivirals yet available and so, for most viral diseases, vaccines are the only cost-effective solution to disease control, apart from some cases where no vaccine is available and slaughtering may be the only control option (e.g. African swine fever), especially when confronted with zoonotic pathogens, as was the case with the Hendra and Nipah virus infections in domesticated species.

Whatever the technology used in their production, vaccines fall into two categories: they are either attenuated or inactivated products. The immunological response in the vaccinated animal depends on whether the vaccine is attenuated or inactivated, with attenuated vaccines generally giving more effective and long-lasting immunity.

Gene-based technologies have already had a major impact in animal vaccinology, the most spectacular example being the demonstration that it is now possible to vaccinate using naked DNA. For the first time this makes it possible to vaccinate animals against viruses that cannot be cultured *in vitro*. Nevertheless, vaccines are not mere scientific concepts, they must work effectively to protect the animal against the clinical signs of the infection or, better still, against infection itself and, clearly, their efficacy does not depend on the level of sophistication used in their production. New vaccines produced using gene-based technology must either show an improvement in comparison with vaccines made using the available technologies, or at least be equivalent in effectiveness to the classical products. The history of vaccinology shows us that each new technology finds its own application niche.

One of the presentations related to the production of a vaccine against East Coast Fever using genomic and post-genomic technologies. Until now, the only available vaccination protocol for this disease was infection with a fully virulent *Theileria parva* followed by treatment to reduce the clinical disease. This is reminiscent of the early days of variolation to protect against smallpox. The availability of safe vaccines as a result of gene-based technologies would be a major improvement in such cases.

To facilitate control programmes, and for economic reasons, it is important that newly designed vaccines used for vaccination campaigns include markers in order to distinguish vaccinated from infected animals. The concept was first tested using DNA viruses whose genomes could easily be manipulated using molecular techniques to produce marker vaccines. Reverse genetics of viral pathogens can now provide new immunogenic constructs that, if properly used, can act as effective marker vaccines for many viruses with RNA genomes. Another important development is the possibility we now have to devise vaccines against invertebrate vectors (ticks, insects, etc.) that are responsible for the transmission and spread of many important human and veterinary pathogens.

Due to the highly dynamic nature of the genomes of many of the pathogens that need to be controlled, and also the genetic polymorphism of vector species where these are involved in transmission, new vaccines may have their limitations.

An important feature to be considered in vaccine design is environmental stability. Several of the newly engineered vaccines (e.g. the vaccinia-rabies recombinant) are sufficiently stable to be used in developing countries, which may have only modest storage and distribution infrastructures.

Finally, intellectual property rights (IPR) and the use of products and procedures already patented is an important issue to consider with any application of genomic-based procedures worldwide, including in developing countries.

For application to vaccine design and production in developing countries, the main recommendations are:

- Vaccines should be multivalent in order to elicit ample protection against a range of related forms of pathogen, wherever antigenic diversity has been documented. For specific geographical areas, it may be necessary to tailor a vaccine according to the antigenic strains encountered in a defined area.
- Special attention should be paid to the stability of the vaccine under adverse storage and delivery conditions.
- IPR issues should be discussed at a specific meeting involving not only scientists but also representatives of industry, patent lawyers and specialists in international trade.

Session III: Gene-based technologies applied to plants, rumen microbes, and systems biology

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This session focused on the role of gene-based technologies in improving nutrient supply and utilization by the animal. The session dealt with three main components: (1) forage nutrient supply, (2) efficiency of digestion by gut micro-organisms, and (3) nutrient-tissue interactions in the animal. Each of these areas was addressed by a series of plenary papers. Research undertaken in each of these areas should be considered in terms of the potential impact on the animal. An integrated approach combining these elements is required to achieve an improvement in nutritional status of animals. For example, attempts to modify the nutritive quality of forage should take into account the effect on efficiency of rumen fermentation and the flow of nutrients for absorption and utilization by the animal. Similarly, optimizing nutrient-gene interactions for production traits must take into account which forages are likely to produce these benefits after they have been digested by gut micro-organisms, and nutrients absorbed by the animal.

Forage nutrient supply

The paper in this component demonstrated that gene-based technologies are well advanced in programmes to develop transgenic forages or use molecular genetics tools to breed and select for forages with specific nutritive qualities. Molecular assisted breeding and transgenics could currently be used to:

- Reduce the concentration of compounds that retard digestion, especially:
 - lignin (in grasses);
 - tannins (in legumes); and
 - toxins (in legumes).
- Optimize the concentration of desirable compounds, including:
 - proteins resistant to rumen degradation;
 - sulphur-containing amino acids; and
 - soluble carbohydrates (e.g. fructose).
- Enhance resistance to diseases and drought.

It was also apparent that applications of gene-based technologies towards these objectives are well advanced compared with animal approaches, which are technically more difficult, have much longer generation times and are affected by more complex ethical issues.

In the forage plant area, current work focuses mainly on temperate species, with little effort in tropical plants, but, comparatively, the effort in forage plants is miniscule compared with grain-based crops for human consumption. One major reason for this bias is that return on investment from grain crops is huge compared with that from forage plants. Also, altering structural characteristic, such as lignin composition, and protective chemicals, like tannins, may compromise survival mechanisms of tropically adapted plants. Substantial progress could be made in improving nutritive value of tropical forages through conventional breeding and selection. However, gene-based technologies would be particularly useful in identifying the genes or QTL associated with desirable nutritive traits. An example of improving nutritive value in a plant used for forage could be molecular marker-assisted breeding for desirable digestibility characteristics in fibrous residues from the major grain crops in the tropics, i.e. rice, sorghum, maize and millet.

Efficiency of digestion by gut micro-organisms

A paper on genomics of rumen bacteria outlined the exciting and rapid progress that is being made in sequencing the entire genomes of representatives of the predominant rumen bacteria. This work is partially completed, and will soon result in publicly available databases containing the annotated sequences from these organisms. However, research will need to continue to assign activity to genes of unknown function, which could represent a large proportion of these genomes. Already it has been demonstrated that the cloned cellulase genes from some of the cellulolytic bacteria only represent a small fraction of the genes encoding for this function based on the genome sequence analysis. During discussion on this paper, several points were emphasized.

- Genomics of rumen micro-organisms – especially fibre-degraders – is of critical importance to developing countries that rely on low-quality roughages and crop residues as basal feeds.
- Genomic studies of fibrolytic bacteria in the short to medium term will be predominantly in developed countries, since the expertise and equipment required will not be available to developing countries in the time frame considered.
- Developing countries will be able to engage in this gene-based technology through bio-informatics studies, since sequence information will be publicly available and accessible to developing country scientists. However, scientists from developing countries currently are not skilled in this rapidly expanding area of biology.
- Practical application of information from such research is currently unclear but could involve industrial production of key lignocellulytic enzymes for pre-treatment of fibrous residues. Also, additional information could be generated from genomics studies that provide insight into the nutrient requirements of rumen and gut organisms that in turn could be employed in practical feeding strategies.

Several papers, including a presentation in another session, identified the greatest current demand and potential application of gene-based technologies in developing countries as being the need for molecular-based tools for studies of gut microbial ecology. During the discussions, it was clear that quantitative methods for PCR and oligonucleotide probing based on analysis of the small sub-unit ribosomal genes are in demand, and already being employed by some scientists from developing countries. These methods are affordable and within the current capacity of molecular biology laboratories in such regions. There was unequivocal

support for the application of molecular ecological tools as they will be essential for developing strategies to:

- reduce methane;
- counter the effects of anti-nutritional factors;
- predict rumen microbial responses to bio-active compounds;
- improve the utilization of varying feed resources through a better understanding of the responses of major functional groups to these nutrients; and
- monitoring gut pathogens associated with issues of food safety.

These tools will need to be employed if nutritional research in the animal sciences is going to make progress in the future.

A paper was presented on the development of a genetically modified pig, which expressed a transgenic phytase into the digestive tract via the saliva. This was an elegant demonstration of the progress that is being achieved in animal transgenesis. These animals were shown to be healthy and could digest and absorb more phosphorus; in addition the gene insert (or trait) was shown to be stable over several generations. The biological success of this project and its potential use in developing countries was overshadowed by the reluctance of several countries to engage in the development or use of transgenic animals. It was also recommended that if transgenic animals gained acceptance in developing countries, then the gene constructs and transgenesis technology should be used to modify locally adapted breeds rather than introduce elite transgenic breeds from developed countries.

The main conclusions and recommendations on transgenics for improved digestion were that:

- limited current applications exist due to consumer and government resistance;
- the genetic constructs should be used in indigenous breeds rather than introducing unadapted high producing exotic genotypes; and
- it was unlikely that there would be any success when using traits governed by gene networks, but the use of single gene effects were feasible (e.g. phytase).

Nutrient-tissue interactions in the animal

One of the papers in this session described in detail the potential impact of nutrient supply on host-tissue metabolism, production performance and on gene expression. This is an emerging and complex area of animal science, but the first experiments are now being conducted on the influence of nutrients on gene expression; on animal tissue function and muscle development; and on the programming of gene expression by the nutritional environment in the developing foetus. The conclusions were that gene-based technologies (e.g. marker-assisted selection) used in combination with conventional breeding and selection programmes could be used to make rapid progress in developing superior phenotypes for production traits. The priority area identified for developing countries was determining the characteristics associated with survival and superior productivity during seasonal nutrient deprivation, which is experienced in many developing countries, especially in the tropics. Issues related to product quality (tenderness, marbling etc.) were not considered currently important for poverty alleviation.

The following strategy was outlined as an approach for developing ruminant animals that show superior resilience during nutrient deprivation:

- characterize locally adapted breeds for “nutritional resilience” using phenotypic attributes, e.g. adaptive behaviour, metabolic rate, muscle and bone structure;
- identify the genes (using QTLs, SNPs) that account for significant variance in weight loss and compensatory growth during periods of nutrient deprivation followed by improved nutrition; and

- consider the possible positive and negative implications of selecting for “nutritional resilience”, e.g. toughness, feed conversion efficiency, and female reproductive performance and lactation.

The need to obtain insight into nutritional influences on growth rate, immune competence, stress responsiveness, reproduction and *in utero* effects was stressed. The potential implications of this rapidly emerging field of research within the agricultural context specifically for livestock production systems, are that the management of the breeding herd, especially during pregnancy, could have long-term impacts on health, growth rate, reproductive efficiency, milk and wool yield of the offspring. All these in turn could have a significant economic impact.

Developing countries would benefit greatly from the development of basic reagents and tools, including protocols for detection, expression and transfer of genes; training; and improvements in laboratory infrastructure to undertake research based on gene-based technologies. An exponential growth in molecular tools in the last decade should not discourage scientists in developing countries from using them to solve problems relevant to the developing world. Promoting the networking of groups in developing countries for research and development work in “modern biotechnology” will bring the necessary impetus for addressing the emerging challenges of providing adequate, safe and good nutrition to livestock, while conserving the environment and utilizing available resources in a sustainable manner.

	Relative cost	Research capacity and progress		Feasibility	Public and government acceptance	Attractiveness
		Developing country	Developed country			
Forage plants						
• Molecular selection and breeding	Moderate	Low	Moderate	High	High	High
• Transgenics	High	Low	Moderate	High	Low	Low
Rumen and gut microbes						
• Ecology	Low	Moderate	High	High	High	High
• Genomics	High	Low	Moderate	Moderate	High	Moderate
Animal nutrition–genetic interaction						
• Marker and gene assisted selection	Moderate	Low	High	High	High	High
• Transgenics	High	Low	Moderate	Moderate	Low	Low
Nutrition programming of gene expression	Moderate	Low	Low	High	High	High

Panel Discussion I: Which gene-based technologies are most likely to succeed in enhancing animal productivity in developing countries?

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The panel focused primarily on three gene-based technologies. Dr Rod Mackie (University of Illinois, USA) described the analyses of specific genes in the microbial flora that populate gastrointestinal tracts and discussed how the expression of these genes can affect the productivity and health of livestock. Dr John Gibson (ILRI, Kenya) discussed the identification and analysis of various genetic parameters of biodiversity and how these parameters can potentially be used to maintain and exploit diversity in livestock breeding programmes. Dr John Egerton (University of Sydney, Australia) described his experience in developing a recombinant vaccine for footrot in sheep and goats, and some lessons learned from its application in the developing world. The conclusions and recommendations from the discussion session following these three presentations are given below.

1. Microbial populations of the gut exhibit a great diversity of organisms, ecology, evolution and function. Many of these gut microbes have yet to be identified and characterized because of difficulties in culturing them *in vitro*. Further understanding of their inter-relationships in the gut and their functions for their livestock hosts is required for improving the nutritional status of livestock in developing countries. Since much of the analysis conducted to date has been on gut microbes of livestock in developed countries, it is recommended that gut microbes of livestock in developing countries be given a high priority for future studies.
2. The breadth of biodiversity amongst livestock breeds existing in the world today is very large and should be taken into account in any long-term breeding programme. In many cases, the inclusion of existing breeds of livestock into a breeding programme will provide greater benefits than breeding for specific traits within a breed. It is recommended that this large diversity of existing traits amongst breeds be considered whenever possible in breeding programmes for livestock in developing countries.
3. New and better vaccines, some of which will come from the application of gene technology, are needed to improve the health and productivity of livestock in the developing world. Finding the correct antigens for these vaccines requires knowledge of the pathogenesis of a wide spectrum of diseases, and so the research involved in identifying these antigens will be long term and costly. Adoption of improved vaccines by poor farmers will depend on containment of vaccine cost. Also, it is essential that animal health infrastructures exist at the local level to ensure accurate disease diagnosis and to disseminate information about the correct use of vaccines in the control of disease. Despite these obstacles, vaccines are a proven way to successfully combat infectious diseases and it is recommended that their development against livestock diseases of the developing world be both continued and accelerated.
4. The gap between “a practical problem in search of a solution” and “a technique in search of an application” can only be closed by those individuals who clearly understand both the problems and the techniques. Hence, meetings such as this symposium, which are attended both by individuals familiar with livestock problems in the field and by scientists actively using gene-based technologies, are essential means of communication. It is recommended that similar symposia be held in future years and that developing

countries around the world continue to be well represented at the meetings. Equally important is the identification of funding opportunities to hold regional meetings (e.g. in West Africa or in East and Central Africa) that bring together livestock scientists of developing countries who share common research objectives.

5. Gene-based technologies are not a "silver bullet" for solving all livestock issues of developing (or developed) countries. They are, however, one of many different approaches to be considered and utilized. The first gene was cloned about 25 years ago and many of the associated technologies are still in their infancy. Ultimately, these technologies have the potential to be of major benefit to owners of livestock in the developing world. Thus, it is recommended that gene-based technologies continue to be considered within the context of the broad spectrum of possible solutions to problems faced by livestock owners in developing countries.
6. Priorities should be determined by the needs of livestock owners in the many different regions and environments where livestock are kept. In each country, policies and procedures should be established for setting such priorities. The challenge and the recommendation of this Panel is to establish mechanisms that ensure gene-based technologies are widely available and properly understood so that they may be incorporated into these policies and procedures when appropriate.

Panel Discussion II and Session IV:

Role of international organizations and funding agencies in promoting gene-based technologies in developing countries

Gene-based technologies in environment, food safety and animal industry, and related ethical and intellectual property right issues

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Actions should follow the procedure of identifying a need and then looking for a solution, not the reverse: "We have a gene-based technique – how can we use it?". This principle of starting with the need is evident in the attitude to using gene-based technologies for biodiversity. If we start by thinking about the technology we are led to conclude that it can be used for genome mapping at the molecular level and thus identify diversity between breeds of livestock. Enormous investment of funds and time would be needed to analyse so many genomes or to select those thought to be critically endangered or similar and to put the results into a database.

The right way is to identify the need, i.e. conservation of livestock biodiversity. Gene-based technology enables us to meet that need without genome analysis. Simply collect and store, in secure locations and by using long-term methods, the cells of each breed, which contain the record of molecular diversity. When the need arises in the future, samples can be sequenced for the differences in bases to be revealed. In this way the objective of conservation is achieved quickly and relatively cheaply.

Identification of needs in developing countries

Parameters that should be considered include:

- poverty;
- reduction of human labour in caring for livestock;

- local energy; and
- improved local food production.

The local socio-economic and cultural values must be included, and address:

- stakeholder involvement in identifying needs and in decision-making;
- cultural sensitivity to understand local values; and
- ethical standards, applicable in both donor and recipient countries.

This action probably needs to be addressed on the basis of groupings of countries or livestock farming systems, or both, as needs will vary.

Design and search for gene-based solutions applicable to the livestock sector

This will require characterization of the broad categories of existing gene-based options for livestock.

- Characterization must recognize the contrast in needs between farmer families and intensive units near urban centres.
- There must be awareness of which technologies are contentious and which are non-contentious.
- Classify existing gene-based technologies according to:
 - opportunities – matching the needs for which they may offer solutions;
 - capabilities – defining the extent to which each technology actually can address an identified need;
 - dangers – recognizing dangers, and whether they are science-based, social-based, economics-based, ethics-based or insensitive to local values;
 - costs, time frames and scale of benefits; and
 - whether local, regional or global in scope.

A prerequisite for the design and search for appropriate gene-based solutions is to identify needs of developing countries, as mentioned above.

The broad categories of existing gene-based options to be characterized include:

- animal health: diagnosis, protection and treatment;
- ruminant and non-ruminant nutrition and metabolism;
- reproduction enhancement;
- transgenic livestock;
- germline manipulation;
- gene-based trait selection;
- molecular analysis of genetic diversity; and
- animal identification and traceability.

This is not a comprehensive list, and each item could be subdivided.

New gene-based technologies suited to identified needs

Novel and currently unavailable gene-based technologies, that might be designed to address some of the identified needs, should be sought. Emphasis should be placed upon simpler gene-based technologies, such as those that are small-scale, cost-effective and use local resources. For example, to provide energy to relieve the heavy labour undertaken mainly by women in caring for livestock.

Special study of genomic impacts upon the environment, food chain and human genome

- Understanding the genetic linkages between species that are involved in the animal food chain when innovative gene-technologies are either introduced or contemplated.

- Clear recognition of the difference in dangers between “substantial equivalence applying to DNA” and the outflows from later-generation gene-based technologies in terms of proteomics and functional genetics when metabolic and physiological changes are the target.
- Documentation of cases in which there are interactions between a transgene and the recipient animal. Several important examples were cited in the Symposium, such as the response of the Boran to trypanotolerant genes from N'Dama cattle.

New approach to risk assessment

- We need to move on from the bland scientific statement that “There is no evidence that ...” – implying that therefore there is no risk.
- Recognition of uncertainty and unknown risks in gene-based technologies must be given a new foundation based upon anticipation and testing, not solely using probabilities.

Probabilities are not good enough when a catastrophe may happen. Fears of unknown and unexpected risks of a science-based nature have been recognized recently by the National Research Council of the National Academies in the USA (Animal Biotechnology: Science Based Concerns. Report available at: <http://www.nap.edu/books/0309084393/html/>) and a similar recent report from the UK makes the same points (Food Ethics Council, UK, 2003, *Engineering Nutrition: GM crops for global justice*. Available at: <http://www.foodethicscouncil.org/library/reportspdf/gmnutritionfull.pdf>).

IPR and TRIPS, and safety regulations for gene-based technologies with animals

Developing countries need assistance in several fields:

- better understanding the present and developing situation regarding IPR and TRIPS for livestock using gene-based technologies;
- obtaining resources and advice on the design of national IPR legislation; and
- designing national legislation for safety, testing and approval of new food products from animals.

Education and training

Training and education in gene-based technologies should focus on younger scientists through network links.