# TABLE OF CONTENTS

# OPENING SESSION: SETTING THE SCENE

**Chairperson: S. Jutzi, FAO**

**TOPIC: Setting the Scene**

# A vision of gene-based technologies for the livestock industries in the third millennium

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While knowledge of DNA structure is now fifty years old, technologies for intervening in genetic structure effectively date back just a decade. From small beginnings with restriction enzymes, we have in the last ten years had an explosion of techniques for isolating, amplifying, reading and inserting DNA. These techniques have been scaled up and automated to make possible mass genotyping. Coupled with developments in information management, this wealth of data facilitates prediction of genetic structure and function, and thus accelerates the pace of knowledge accumulation. This explosion in knowledge has been compared in significance to the development of the Periodic Table one hundred years ago, and to the expansion of horizons to encompass the whole globe in the 16$^{th}$ century [1,2].

The first complete genome sequence of an organism was for yeast in 1996. Since then, the much larger task of doing a complete human sequence has been completed. Those of all domestic animals are following rapidly. It will always be impossible to foresee the full potential, both positive and negative, of such an explosion in knowledge. However, already aspects of gene-based technologies are beginning to have an impact in the livestock sector.

The first, and most obvious, concerns the feed supply, which constitutes 50–75% of total costs in many livestock systems. Production costs for corn and soya bean are being reduced by genetic modification of the crop for herbicide and insect resistance. Corn has also been modified with the effect of reducing phosphorous and nitrogen excretion in swine and poultry, and also to provide more valuable amino acid balance.

Genetic modification of the animal is also possible. Most dramatically, the insertion of the grown hormone DNA in fish accelerates growth. However, in this and all other cases the genetic modification of animals had produced profound physiological disturbances. On the other hand, the administration of GM-produced growth hormone to dairy cows in now routine in the US and several other countries. This is not permitted in Europe, where the attitude to all GM technologies has been much more cautious.

Control of disease in animals using GM technologies is much less contentious. As pressure to reduce antibiotic and drug use increases, genetically modified vaccines with improved specificity and distinguishable from natural infections are already in use. DNA typing is helping with rapid and precise diagnosis. In addition, the interaction of some pathogens (e.g. scrapie) with the genotype of the animal calls for the application of DNA technologies.

Following the BSE epidemic in Europe, safety of livestock-derived foods is high on the research and regulatory agendas. DNA techniques are already in use for tracking of sources of salmonella and E.coli outbreaks, as well as for traceability of product in the food chain.

The possibilities of genetically improving animals for disease resistance or production traits is being pursued along two parallel tracks: using marker technology to augment normal selection programmes, and using functional genomics to target DNA sequences of known or suspected function.

Finally, animals have been genetically modified to contribute directly to human health through the production of therapeutic proteins in their milk, or to produce compatible tissues for human transplants.

Not all of these developments will find their place in livestock production systems, for reasons of cost, consistency, ethics or public acceptability. However, they present such an array of possible gains in health and productivity that we can speak of a revolution in livestock technology.

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**TOPIC: Setting the Scene**

# Challenges and opportunities for controlling and preventing animal diseases in developing countries through gene-based technologies

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The livestock revolution so robustly and frequently described in the past five years, is argued to provide a real opportunity for the rural livestock keeper in developing countries to escape the poverty trap, move away from subsistence farming and enter the more rewarding areas of farm enterprise and income generation. To do so though, will require more than merely acknowledging this marketing opportunity. It will be essential to address the many constraints and critical risks that constantly face rural farming in developing countries. Of these, livestock disease rates as one of the most challenging. However, for effective participation in the livestock revolution it will be essential that livestock disease is either controlled or prevented.

For the livestock producer in developing countries, many of the life threatening diseases that have been eradicated from the developed world area are ever present and the extent and range of production-limiting diseases are considerable. The situation is further compounded since in many cases veterinary services and other animal health delivery systems are either non-existent or ineffective. For some time donor organisations have been driving countries in transition to privatise services such as animal health delivery. The current situation is the virtual elimination of functioning State veterinary services without replacement by a private system – and certainly not in rural areas.

The elimination of the major killer diseases of livestock in the developed world was achieved, for the most part, through considerable State investment, extensive veterinary input and a large share of public money. Such resources are certainly not available today in most developing countries. No wonder therefore that diseases such as Contagious Bovine Pleuropneumonia, African Swine Fever ad Foot and Mouth Disease continue to exist endemically in most poorer regions of Africa and elsewhere.

In terms of the production limiting diseases, control of these in most developed countries is through a mixture of management and therapy. The former requires knowledge and considerable local understanding and the latter resources and supplies. Both of these are limited in the developing country situation, particularly in a rural setting.

Finally but of equal importance, is the considerable negative impact of the presence of disease on trade. Those trading internationally in livestock and livestock products are demanding a clear demonstration of freedom from an ever increasing list of diseases. Participation within national markets is increasingly constrained by the need to provide safe products to urban communities. But in the absence of effective surveillance it is often not possible to determine what disease is present, let alone meet the rigours of demonstration of freedom from a particular disease or infectious agent.

Given this complex of challenges, can gene-based technologies really make a difference to the management of livestock disease for the producer in developing countries? To be effective in the developing country situation, any intervention must be relatively simple, cost effective, sustainable and convincing. Can this be delivered? Perhaps an insight can be gained from an appreciation of the fundamental nature of gene-based technologies. Inherent in the approach is the recognition that the gene is the basic building block of biology. Management and manipulation of the gene therefore enables us to design and direct an endless array of precise solutions, whether this be designer livestock, genetically engineered biological products or genetically altered organisms.

Without doubt, the availability of livestock resistant to disease, or at least one or two of the major diseases affecting livestock in a particular region, is a simple and applicable solution to the developing country situation. Attempts to understand the genetic basis of trypanotolerence are still on-going but if successful would enable livestock production in large areas of Africa currently restricted by the presence of trypanosomosis. Another example would be the demonstration of resistance to internal parasites by certain breeds of sheep. Locating the genetic basis of this could be revolutionary in the management of this particular disease risk. As work starts on sequencing both the bovine and the ovine genome, the future opportunities for designing livestock resistant or tolerant to a range of diseases looks highly promising.

Looking at the causative agents of livestock disease, the ability to exquisitely alter these to better understand the way they cause disease is providing a fast track to developing ways of control or eradication. For example identifying the gene coding for a protein that allows cell attachment would permit genetic engineering to delete this gene from a particular disease causing organism. This could then form the basis of a vaccine that is safe but highly efficacious. Even more exquisite is the incorporation of this particular gene into another carrier such as harmless virus, bacteria or other similar organism. The expression of the protein can then be used to evoke an immune response in a susceptible host without any risk of disease. Many groups around the world are currently exploring these concepts for a wide range of causative agents and a variety of different expression systems.

All the above is already feasible but not without problems. Firstly and perhaps foremost, the acceptance of genetically modified organisms (GMOs) by consumers is far from complete. Considerable debate has taken place in the plant industry and whilst partially applicable to animals, the issues are dissimilar in many areas. In developed countries a great deal of research is taking place in this area but it is still unclear how well the consumer will accept genetically modified animals as a food source, or products from animals protected by genetically engineered vaccines. This debate needs to urgently take place. Secondly, this technology is not without considerable cost. If it is to be harnessed for those diseases that most affect rural livestock producers in developing countries then a new paradigm of global research partnerships and funding will be required. There will need to be a recognition that those diseases that continue to affect livestock in the developing countries, will continue to pose a risk to all livestock. Their eradication or control in the developing world will be an advantage to all livestock producers. Their continued presence in certain regions might seem to be an advantage to producers free of disease but recent outbreaks of Foot and Mouth Disease in Europe clearly demonstrate the fallacy of this approach.

Gene-based technologies have the potential to deliver workable solutions to the management of animal diseases and these can be considered particularly applicable to the developing country situation. Success though will depend on consumer acceptance of this approach and the use of innovative global partnerships to undertake the enabling research.

# SESSION I: GENE-BASED TECHNOLOGIES APPLIED TO LIVESTOCK GENETICS AND BREEDING

**Chairperson: J. Gibson, ILRI, Kenya**

**TOPIC: Gene-based technologies applied to livestock genetics and breeding**

# Molecular genetics and livestock selection: Approaches, opportunities and risks

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There are over 1,200 million cattle worldwide that provide a source of food, motive power and clothing. Cattle were first domesticated about 12,000 years ago with both the archaeological [1] and molecular evidence [2,3] suggesting that this occurred in the Near East and that domesticated cattle then spread to Africa and Europe. Traditionally breeding was carried out at a local level, often using a limited number of shared bulls. The selection of individuals with particular characteristics suited to local environments, needs and preferences led to the emergence of distinct breeds with characteristic phenotypes. In 1993 there were 783 cattle breeds worldwide [4], although the definition of a breed is often vague.

With the introduction of artificial insemination (AI) in the more developed countries during 1950s particular bulls with desirable characteristics were more widely used in preference to local bulls. The use of AI, coupled with improvements in management in Europe and North America, allowed rapid progress to be made in the improvement of simple production traits. Breed improvement has been further enhanced by the development of statistical methods to maximize genetic gain achieved by selection on traits that can be readily measured. Consequently, where the economic environment supports high input agriculture, there has been a dramatic increase in milk yield and meat produced from the improved stock. The unfortunate consequence of intensive selection in these areas has been the reduction of genetic diversity, both within the selected breeds, as the superior individuals within these breeds have been used as breeding stock, and also through the replacement of traditional breeds. While the use of improved breeds in areas advantaged by good environmental conditions and a favourable economic climate has allowed the increase in production, all-be-it with the penalty of lost diversity and damage to the environment occasioned by intensive farming practices, in less developed and environmentally less favoured areas the use of these breeds presents a greater cause for concern. Local breeds are usually adapted to survive in their local environments eg with increased tolerance of extremes in temperature or in the face of particular disease or parasite challenge. Attempts at the inappropriate and/or unmanaged introduction of improved dairy breed into some areas has met with disastrous consequences. In 1993, 112 of the 783 cattle breeds worldwide were at risk of extinction. The greatest risk is the replacement of local stock that are adapted for survival in the face of disease challenge with disease sensitive stock in areas where standards and resources to provide extensive veterinary care are not available.

Much work has been carried out over the past 10 or so years to produce genetic and physical maps of the bovine genome [5,6,7]. In the first instance these maps were composed predominantly of anonymous markers, but more recently genes, and expressed sequence tags (ESTs) have been added to the genome maps of cattle. Use of genetic maps together with other molecular genetic approaches, like micro-array technology to examine gene expression, will enable the genes having a major influence on a wide range of traits to be identified. Most

production-associated traits are under the control of several genes, which have varying levels of effect on the trait, and are generally referred to as Quantitative Trait Loci. To date considerable success has been reported in localising QTL for a wide range of traits [8,9], however two notable successes have identified the major genes involved in increased muscling [10,11] and milk production [12].

Knowledge of the loci controlling individual traits will allow the direct selection for favourable alleles at these loci. In the first instance this can be done by marker-assisted selection with markers linked to the gene involved in the trait. However, ultimately, knowledge of the allelic variation within that gene will allow more efficient selection to be carried out. There are several advantages of using markers in selection programmes, rather than relying on phenotype based selection. In using markers it will be possible to introgress favourable alleles for particular traits from one breed into another, taking advantage of specialised characteristics of different breeds, for example to maintain disease resistance while increasing production. By using information on the markers spanning the genome, as well and the genes under selection, it will also be possible to maintain the widest possible genetic diversity within breeds. Thus considered and well-managed use of molecular information will help preserve the genetic diversity of cattle populations.

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**TOPIC: Gene-based technologies applied to livestock genetics and breeding**

# First report on the state of the world's animal genetic resources: Views on biotechnologies as expressed in country reports

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The Food and Agriculture Organization of the United Nations (FAO) has been requested by its member countries to develop and implement the Global Strategy for the Management of Farm Animal Genetic Resources. The global livestock sector is faced with the challenge of the fast increasing demand for animal products in developing countries. FAO has estimated that demand for meat will double by 2030 (2000 basis) and demand for milk will more than double in this 30-year period. On the other hand, animal genetic resources worldwide are disappearing rapidly. Over the past 15 years, 300 out of 6000 breeds identified by FAO have become extinct. Successful genetic improvement programs in adapted indigenous breeds are less than a handful. Although in many developing countries there have been considerable efforts in training professionals in animal genetics, breeding programs applied to livestock under low input farming systems have largely failed. As part of this country-driven strategy for the management of farm animal genetic resources, FAO has invited 188 countries to participate in the First Report on the State of the World's Animal Genetic Resources, to be completed before 2006. To date 145 countries have accepted to submit country reports. The drafting of the country report is under way, with target date August 2003. It must be stressed that the national reports are basically strategic policy documents, and as such the FAO-provided blueprint, approved by FAO's governing bodies, is designed to answer three questions regarding the countries animal genetic resources: where the country is now, where it needs to be, and how to get there (FAO, Animal Genetic Resources Information, 30, 2001; www.fao.org/DAD-IS). The country reports should reflect problems, needs and opportunities. They are organized in five parts. Part 1 reports on the state of genetic resources in the farm animal sector covering both *in-situ* and *ex-situ* conservation aspects, as well state of the art of techniques being used, in the context of production systems and socio-economic conditions of each country. Part 2 should describe the changing demands on the farm animal sector and the implications for future national policies in conservation and utilization of animal genetic resources. Part 3 is a review of the state of national capacities related to farm animal genetic resources and an overall assessment of capacity building requirements. Part 4 should identify national priorities covering diverse fields of activity, animal species and breeds, as well as short and long term needs for institution-building, research, information systems, policy, legislation and regulations. Part 5 deals with recommendations for international cooperation, indicating the areas, levels and mode of cooperation which the country wishes to follow, and proposed contributions and requirements. It is expected that countries will include views on biotechnologies in relation to farm animal genetic resources in their reports, within the context of the recommended report structure, particularly in parts 1 and 4. The analysis of country reports may also serve to estimate the gaps in biotechnology application between developed and developing countries.

In September 2003, 41 country reports had officially been submitted to FAO. For this paper, 30 country reports representing all regions were analysed with regard to information on

biotechnologies used in animal breeding and reproduction, in conservation of animal genetic resources and for commercial uses. In addition, the information gained from discussions in regional workshops in Latin and Central America, covering 20 countries, was included.

The West Africa region was represented by 12 country reports. With few exceptions, all use AI (artificial insemination), mostly in cattle, but at a very low percentage. No ET (embryo transfer) is used, and limited molecular characterization has been carried out, mainly as part of international development projects. Priorities were expressed in capacity building and training on AI and ET in the context of performance and genetic evaluations of livestock, and also in molecular techniques for the characterization of local animal genetic resources. Major constraints to reach priorities are financial resources and the lack of skilled human resources to undertake in-country training.

Eastern Europe was represented by seven country reports. AI is widely used for several species, mainly cattle, and often connected to national AI programmes and activities of breeders associations. ET is used in a limited number of cases, or it is in national plans. Many countries have legal instruments to regulate AI and ET. Most countries undertake some research on molecular characterization of local breeds. Some countries have gene banks, which often still need further development. Priorities identified are: gene banks, technical expertise in AI, ET and cryoconservation, and the major limiting factor mentioned is availability of financial resources.

Countries of Western Europe, represented by eight country reports, have national AI programmes in place and AI is used widely throughout the farming sector. There are national and private gene banks in all countries for both commercial and conservation purposes. Priorities expressed are in cryoconservation of genetic material, expansion of gene bank activities, breed characterisation (phenotypic and molecular), and adaptations required in national and international policies on the use and conservation of genetic material.

The Near East was represented by only one country report. AI is mostly used in cattle, no ET is used, and work is in progress regarding regulation on the use of GMOs (genetically modified organisms). Priorities were expressed on creation of ET facilities, training in new biotechnological methods and establishment of gene banks. Major constraints are funding and the lack of skilled human resources.

Two countries in Asia reported that AI is used but no ET facilities exist. Priorities were expressed in training, expanding the national gene pool, and updating existing regulations for conservation AnGR. China reported use of AI and ET, and microsatellite DNA technology. No specific priorities were mentioned but the country is implementing plans for a centre for AnGR germplasm.

As a result of a regional meeting on country report preparation, national and regional priorities, ten countries of Central America and the Caribbean, and Mexico identified AI as means of diffusion of genetic improvement, and some mentioned ET as rarely used but of interest. Emphasis, both as needs and actions was put on molecular characterization and cryoconservation of local breeds, especially criollo cattle, sheep and goats. All countries identified the need for national programmes in conservation and utilization of AnGR, including development of biotechnology and updated legislation. A regional meeting with ten South American countries identified similar interests and priorities in biotechnology.

From country reports analyzed to date by FAO, it is concluded that AI is the most common biotechnology used developing countries and needs are expressed for training and expansion.

Often AI is introduced without proper planning and is seen as a potential threat to the conservation of local breeds. Although ET use is mentioned and the desire for its introduction or expansion expressed, no clear objectives for this technique are mentioned. All countries have expressed a wish for the introduction and development of molecular techniques, often as a complement to phenotypic breed characterization. Cryoconservation was identified as a priority by all countries and gene banks were recommended, but at the same time funding remains a major constraint. When GMOs are mentioned it is mainly to express the lack of proper regulations and guidelines for their eventual production, use and exchange. It is, however, not clear in all cases whether the technologies used are a sensible part of an overall genetic improvement strategy.

**TOPIC: Gene-based technologies applied to livestock genetics and breeding**

# Development of germline manipulation technologies in livestock

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Breeding, based on conventional selection, has been the mainstay for livestock genetic improvement for more than 70 years, and is still so today. Sophisticated statistical and computing tools now enhance conventional genetic selection, nevertheless traits such as fertility and disease resistance have still proved difficult to improve. Gene transfer technology (transgenesis) offers the potential, as yet unproven, to modify these types of traits.

A transgenic animal carries integrated DNA sequences in its genome. The introduced DNA can be derived from species other than the host and can be modified *in vitro* prior to being introduced into the germline. Therefore, transgenic livestock overcome some of the limitations of classical animal breeding regimes, where importation of genes by crossbreeding is limited to those traits already present within a given species.

The most used method for introducing genes into the germline of animals involves the direct microinjection of DNA into the pronuclei of fertilised eggs. Pronuclear microinjection, although conceptually simple - a fine needle is used to pierce the pronucleus and the DNA is injected - requires special equipment and considerable dexterity on behalf of the person involved. By adapting the techniques employed for gene transfer in mice, pronuclear microinjection has been used to generate transgenic farm animals.

The first attempts to genetically modify livestock owe much to pioneering experiments in mice, where the introduction of growth hormone gene dramatically increased the growth rate and final size of the animals. By contrast, the same approaches in livestock did not prove successful. Indeed, in terms of modifying livestock for agricultural purposes, most of the early expectations were not realised. Rather, it has been the development of novel uses of livestock, particularly for human medicine, that has led the way and advanced this technology. For example, targeting the expression of human proteins to milk and generating animals for organ transplantation (xenotranplantation).

The majority of transgenic livestock have been produced using this method but it only allows gene addition. For gene removal the integration of the introduced transgene has to be targeted to the gene of interest. This requires a relatively high frequency of homologous recombination that occurs in embryonic stem (ES) cells; it does occur in somatic cells but is substantially less efficient. ES cells once introduced into an embryo can contribute to all cells types of the adult animal. Thus the desired genetic modification can be identified and selected for while the cells are grown in culture. This enables vastly more sophisticated genetic changes to be engineered, including gene knock-out. Unfortunately, ES cells have only been isolated for mice and even in this species there are only a few permissive strains.

The lack of methods for gene knock-out in livestock was the driving force leading to the development of nuclear transfer technology. This technique was made famous through the generation of ‘Dolly’. It is fair to say that although catching both the scientific and media in a

frenzy of cloning issues, perhaps the greatest legacy of nuclear transfer will be the development of cell based therapeutic strategies based on stem and somatic cells to treat human genetic diseases. Although 'Dolly' is not herself transgenic, this technique does offer the potential to make transgenic animals more efficiently than by using the pronuclear microinjection method. This is primarily because all founder animals are transgenic and a flock/herd of clonal animals can be produced within one generation. More importantly, nuclear transfer uses cells grown in culture therefore, for the first time, allows precise changes to the germline of ruminants to be attempted.

This has now been shown to be possible, a sheep carrying a disruption of the PrP gene, a targeted insertion into the collagen gene and pigs that have a deletion of the $\alpha$(1,3)galactosyltransferase gene having been produced. However, the generation of knock-out transgenic livestock is a hugely demanding technical and financial undertaking. First, the techniques utilised efficiently in mice, do work in livestock cells but are considerably less effective. Second, the stringent selection and extended *in vitro* culture required for targeting somatic cells (the target for the genetic modification prior to nuclear transfer) reduces their developmental potential, compounding the high cost and low efficiency of nuclear transfer. Finally, nuclear transfer in livestock is beset by the losses *in utero* and after birth, having both a welfare and economic cost. In summary, yes we can generate gene knock-out livestock using nuclear transfer but unless there is a conceptual leap in our understanding of the technique it will not become common place.

At the beginning of last year a new approach to transgenesis was reported for the generation of transgenic mice and rats. Two groups demonstrated that lentivirus (specialised retrovirus) vectors can be used to efficiently introduce foreign DNA into the germline. There appears two dramatic advantages to this technology which make it very appealing for use in livestock. Only a fraction of the resources needed for conventional pro-nuclear injection would be required, given the DNA transfer efficiencies reported in the mouse. Even more appealing is the simplicity of delivery, abrogating the need for specialised equipment. If this method is applicable to livestock – and there is no reason to think it will not be – then previously only dreamed of transgenic applications may become reality, creating tremendous opportunities for the genetic modification of livestock.

Perhaps the most exciting goal envisaged is the engineering of resistance to infectious disease, through combining the efficiency offered by lentiviral vectors with the emerging molecular tool of RNA interference (RNAi which is based on siRNA molecules). We anticipate the generation of transgenic animals that constitutively express RNAi vectors targeting knockdown of a pathogenic virus and/or its transcription products, thereby engineering cellular resistance to infection. These animals will be of great value in dissecting disease progression, the challenge will be to evaluate their potential in commercial breeding regimes.

It is over 18 years since the first demonstration that transgenic livestock can be produced. Subsequently, the development of nuclear transfer technology was set to revolutionise this area of biotechnology, since it overcame the lack of livestock ES cells. Certainly it does enable gene-targeting approaches to the generation of transgenic livestock to be performed; although this produces a recessive mutation in the first instance. Both pronuclear injection and nuclear transfer are inefficient methods for modifying the germline of animals. The recent development of new methods of transgenesis based on viral vectors again offers an avenue to overcome the current restricted application of transgenesis in livestock. Perhaps we are now at the start of a new era in livestock transgenesis?

**TOPIC: Gene-based technologies applied to livestock genetics and breeding**

# Polymorphism in Sahiwal breed of zebu cattle revealed using synthetic oligonucleotide markers

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Livestock improvement greatly depends on the exploitation of DNA level polymorphisms. Specific sequences of DNA are being used as genetic markers to identify loci responsible for expression of complex traits both in man and animals. Presently several classes of markers are available namely RFLPs, AFLPs, VNTRs, STRs, SNPs etc. DNA Sequences with basic repeat motifs of two to six nucleotides can be synthesized and hybridized to genomic sequences from a variety of species to produce multilocus band patterns. Several such oligonucleotide sequences have been reported to be useful in producing highly polymorphic DNA fingerprints in a variety of species. These markers have short-range uses such as parentage determination, individual identification, detection of twin zygosity, etc., and long-range applications such as gene mapping and marker assisted selection

The degree of polymorphism elucidated from a probe or a marker may differ from species to species depending on probe-species combination. It is important to screen DNA markers for their informativeness and polymorphism for various domestic species of animals before considering them for further use. In literature several synthetic probes having the core sequences of (AT) (GT), (GC), (CAC), (GAA), (GGAT), (GACA), (TGG), and (GATA) have been reported for DNA fingerprinting of a variety of species of animals. However, the indigenous Zebu cattle, which constitute major proportion of Indian cattle population has poorly been explored with DNA-based markers. In this study, four different oligonucleotide markers were screened for their usefulness as markers in Zebu cattle.

The investigations were carried out on genomic DNA of randomly selected unrelated (15 animals) and from two sire families (11 animals) of Sahiwal breed of Zebu cattle maintained in a herd at National Dairy Research Institute, Karnal. Oligonucleotide probes were custom synthesised and used after radio-isotopic labelling with ($\gamma^{32}$P) dATP $^{32}$P using the enzyme polynucleotide kinase by the standard procedure. Hybridization of labelled oligonucleotide probes to genomic DNA on Nylon membranes was carried out at 45°C for probes $(GTG)_5$ and $(TCC)_5$, 43°C for $(GT)_8$ and 65°C for $(GT)_{12}$. Post-hybridization treatments and autoradiography were carried out and size of each fragment on X-ray film, i.e. DNA fingerprint, was estimated using computer software GelBase (UVP, UK). Number of total bands and shared bands in the fingerprints of each individual were recorded in the range of 2.5 to 23.0 KB. Number of bands, average band sharing rate (BS), mean allelic frequencies (a) and heterozygosity (h) level were calculated.

All four probes used produced multilocus fingerprints with differing levels of polymorphism. Means of number of bands per individual, band sharing rate, allele frequencies and heterozygosity was calculated. The probes $(GT)_8$, $(GT)_{12}$ and $(TCC)_5$ produced fingerprinting patterns of medium to low polymorphism whereas the probe $(GTG)_5$ produced highly polymorphic pattern. The probe $(GT)_8$ probe produced as many as 32 bands in resolvable

portion of the gel. However, nearly 40% of the bands were shared by all the individuals hence, the average bands sharing rate was found to be high. High band sharing rate in this study indicate that the animals examined might be genetically more homogeneous with respect to $(GT)_n$ sequences. Comparison of average number of bands obtained between different probes reveal that the probe $GT_8$ hybridized to more number of fragments than the other probes. This result indicates that $GT_n$ are more abundant in zebu cattle genome compared to other sequences studied. The probe $(GT)_{12}$ produced a multilocus fingerprints with lower level of polymorphism in comparison with $(GT)_8$ fingerprints. Mean number of bands and polymorphism were low as compared to $(GT)_8$ fingerprints. Variation in the nucleotide constitution of repeat sequences and differences in hybridization and stringency conditions could be the reason for variation in banding pattern of same core sequences of differing length. The probe $TCC_5$ produced multilocus polymorphic fingerprints. The level of polymorphism was low as revealed by high mean band sharing values of 0.75. The reason for this deviation from the present observation could be the variation between genome of *Bos taurus and Bos indicus.* Alternatively it is possible that H*inf*I restriction sites are adjacent to $TCC_n$ sequences are conserved while there may be variation in H*ae*III restriction sites. The probe $GTG_5$ produced highly polymorphic DNA fingerprints. The number of bands ranged between 9-17 with average band sharing of 0.48. The probe $GTG_5$ or its complementary sequences $CAC_5$ produced highly polymorphic fingerprints. High heterozygosity level obtained in this study and low level of mutation rate associated with the sequences indicate that the probe can be used for analyzing population structure, parentage verification and as a marker to identify loci controlling quantitative traits, disease resistance, fertility etc.

**TOPIC: Gene-based technologies applied to livestock genetics and breeding**

# Genetic diversity and differentiation of Mongolian indigenous cattle populations

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Livestock production plays an important role in Mongolian economy. Over the last decade it has contributed to around 80–90% of the gross domestic agricultural products and to 30% of the revenues generated from exportations [1]. Cattle is one of the five tranditional and most important livestock species of Mongolia together with horse, sheep, goat and camel. Out of a total of 1.57 millions Mongolian cattle, 1.55 millions supposedly belong to three indigenous *Bos taurus* cattle breeds, namely Mongol, Selenge and Khalkhun Golun, all herded under extensive pastoral systems. Indigenous Mongolian cattle are generally small but look sturdy and strong. They have a well-off coat of hair, solid forward looking shoulders and short stubby snouts, and they are used for meat, milk and transport. Beef production contributes to 30% of the total meat supply in Mongolia. The Mongol breed is by the far the commonest with 1.53 million animals and it is found almost throughout the country. The Selenge breed, found in Selenge province and numbering 9000 heads, was developed in middle of the 20$^{th}$ century by crossing the Kazakh Whiteheaded with the local Mongol cattle. The Khalkhun Golun breed was developed from local Mongol cattle and it is distributed in Eastern and Sühbaatar provinces with about 10,000 heads [2] Until now, to the best of our knowledge, only a single population of Mongolian cattle has been studied with microsatellite DNA markers [3] and no information is available on the genetic relationship between the Mongolian indigenous cattle breeds.

In this study, we collected samples from two populations of the Mongol cattle (sampled at Ikhtamir soum in North Hangay province and Tsogt soum in Govĭ Altay province) and one population of the Khalkhun Golun cattle (sampled at Tumentsogt soum in Sühbaatar province). Samples were characterised with nine microsatellite markers *MGTG*4B*, ILSTS*005*, ILSTS*006*, ILSTS*008*, ILSTS*023*, ILSTS*028*, ILSTS*036*, ILSTS*050 and *ILSTS*103. To assess the genetic diversity and relationship of Mongolian cattle populations with breeds from neighboring countries and exotic breeds, data from the ILRI cattle genotyping database were included. More particularly, we used previously obtained data from Asian taurine (Hanwoo, Yanbian and Japanese Black), two European taurine (Friesian and Charolais), two African taurine (Baoulé and N'Dama) and two zebu breeds (Sahiwal and Ongole). For each breed, observed (*Ho*) and expected (*He*) heterozygosities as well as the mean number of alleles (MNA) across the nine loci were calculated (TABLE I) [4] between pairs of populations were also estimated and a UPGMA tree was constructed (Figure 1).

The heterozygosities (*Ho* and *He*) in Mongolian cattle populations are similar to those obtained in Northeast Asian taurine breeds but the values are higher compared to the ones obtained for the European and African taurine breeds. The Mongol cattle in North Hangay has

the highest corrected MNA value (all animals or 28 animals only). The UPGMA tree, built with the Reynolds' genetic distances, shows all six Northeast Asian cattle populations clustering into one group linked to the two European taurine breed. Interestingly, the two populations of the Mongol cattle are not closely related to each other. However, bootstrap values between the Northeast Asian taurine breeds, with the exception of the bootstrap value between Yanbian and Hanwoo, are relatively low, therefore the relationship between the Northeast Asian populations should be taken with caution. *Fst* values between the three Mongolian cattle populations are significant ($P < 0.01$), with the Govĭ Altay population being more differentiated from the North Hangay population than from the Khalkhun Golun breed (data not shown). Our data suggest that the traditional classification of Govĭ Altay and North Hangay populations as one breed, the Mongol cattle, should be revisited.

TABLE I. HO, HE AND THE MNA ACROSS THE NINE LOCI

| Populations | Countries | Number of samples | Ho ± SD | He ± SD | MNA ± SD (all animals) | MNA ± SD[1] (28 animals) |
|---|---|---|---|---|---|---|
| North Hangay | Mongolia | 44 | 0.726 ± 0.032 | 0.641 ± 0.026 | 7.78 ± 2.28 | 7.09 ± 2.14 |
| Govĭ Altay | Mongolia | 40 | 0.639 ± 0.056 | 0.619 ± 0.026 | 6.11 ± 2.52 | 5.63 ± 2.37 |
| Khalkhun Golun | Mongolia | 40 | 0.673 ± 0.038 | 0.658 ± 0.026 | 6.44 ± 2.13 | 6.03 ± 2.09 |
| Hanwoo | Korea | 77 | 0.635 ± 0.051 | 0.699 ± 0.020 | 7.00 ± 2.45 | 5.48 ± 2.07 |
| Japanese Black | Japan | 30 | 0.673 ± 0.037 | 0.644 ± 0.031 | 6.56 ± 2.01 | 6.38 ± 1.95 |
| Yanbian | Northeast China | 30 | 0.635 ± 0.041 | 0.639 ± 0.032 | 6.00 ± 2.24 | 5.85 ± 2.20 |
| Friesian | Netherlands | 35 | 0.644 ± 0.036 | 0.632 ± 0.028 | 5.78 ± 2.33 | 5.61 ± 2.29 |
| Charolais | France | 33 | 0.604 ± 0.035 | 0.594 ± 0.029 | 4.67 ± 1.94 | 4.57 ± 1.90 |
| Baoulé | Burkina-Faso | 35 | 0.547 ± 0.047 | 0.506 ± 0.029 | 4.56 ± 1.88 | 4.34 ± 1.82 |
| N'Dama | Guinea | 35 | 0.509 ± 0.053 | 0.535 ± 0.029 | 3.89 ± 1.45 | 3.77 ± 1.39 |
| Sahiwal | Pakistan[2] | 35 | 0.695 ± 0.037 | 0.656 ± 0.027 | 6.11 ± 1.27 | 5.89 ± 1.28 |
| Ongole | India | 28 | 0.752 ± 0.026 | 0.657 ± 0.031 | 5.78 ± 1.39 | 5.78 ± 1.39 |

[1] Mean value after 250 re-sampling; [2] Sampled in Kenya.

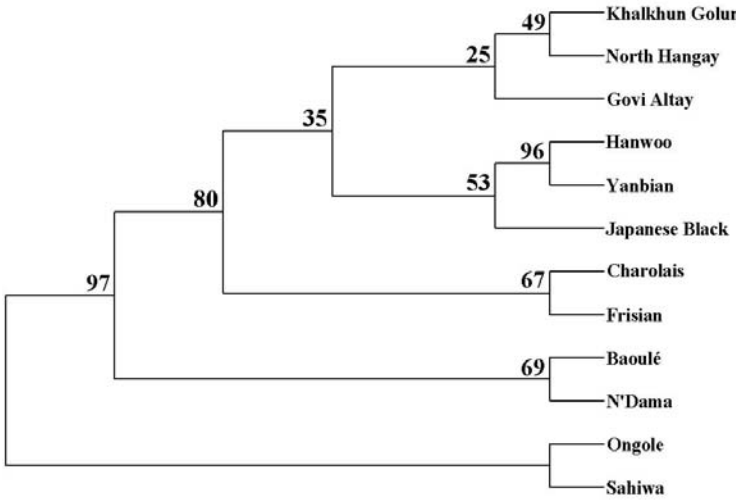


*FIG.1. UPGMA tree, constructed with the Reynolds' Fst genetic distances, showing the relationships among the 12 cattle populations. Numbers indicated bootstrap values in percentage after 1000 resampling. Sahiwal and Ongole were used as outgroup.*

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**TOPIC: Gene-based technologies applied to livestock genetics and breeding**

# Genetic diversity and relationships of Vietnamese and European pig breeds

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East Asia contains more than 50% of the world's pig population and Europe about 30% (according to FAO inventory [1]). Both indigenous resources were domesticated from different sub-species and are assumed to be the basis of the world-wide genetic diversity in pig. Indigenous resources of Asia, however, are less defined and only rarely compared with European breeds. Taking advantage of DNA diagnostics, animals within as well as between breeds from Vietnam and Europe were analysed for numerous well defined markers in order to gain more knowledge about pig genetic biodiversity. The main objective was to investigate indigenous Vietnamese pig breeds from different local geographic regions.

A set of pig breeds was chosen for this study of genetic diversity: five indigenous breeds from Vietnam (Mong Cai, Muong Khuong, Co, Meo, Tap Na), two exotic breeds kept in Vietnam (Large White, Landrace), three European commercial breeds (Pietrain, Landrace, Large White), and European Wild Boar. Samples and data from 317 animals (17 to 32 unrelated animals per breed) were collected. A panel of 27 polymorphic microsatellite loci was chosen according to FAO recommendations for diversity analyses and genetic distance studies. The loci were distributed evenly over the porcine genome with additional loci linked to immunological relevant genes (MHC, IFNG). Moreover, a few Type I loci (RYR1, FSH) were genotyped. DNA was isolated and PCR fragment lengths analysis were carried out on an ALF DNA sequencer (Pharmacia, Freiburg, Germany). Some of the RFLPs were analysed by agarose gel electrophoresis. Selected microsatellite alleles of equal lengths were sequenced for animals of different breeds.

Within-breed diversity estimated heterozygosities and tests for Hardy-Weinberg equilibrium by taking into account sample sizes, tests per locus and breed as well as breed-locus combinations. Calculations were performed using the BIOSYS-1 software package [2]. Breed differentiation was evaluated by the fixation indices of Wright [3]. Genetic distances between breeds were estimated on the basis of allelic frequencies of the loci in each breed using different measures, e.g the standard Nei's distances. Distances between breeds were further analysed according to the neighbour-joining algorithm of Nei [4] and the bootstrapping procedure of Felsenstein [5].

In average of the marker loci, heterozygous genotypes occurred more frequently than expected, but this was, not statistically significant. Heterozygosity was higher in indigenous Vietnamese breeds than in the other breeds.

Breed differentiation was shown which allowed grouping of all individuals in clusters corresponding to the breeds. Herein the Vietnamese indigenous breeds form a distinct cluster with considerable genetic distance to the European breeds. Vietnamese exotic breeds were similar to the breeds in Europe. European Wild Boar displayed closer relation with commercial breeds of European origin than with the indigenous Vietnamese breeds.

The microsatellite loci which are closely linked to functional genes of immune response showed differences between breeds. This finding may indicate adaptation to local geographic conditions. Type I loci revealed considerable differences between Vietnamese and European breeds which are partly due to breeding influences.

The comparative DNA sequencing showed differences between microsatellite alleles of equal lengths. About 30% of these alleles displayed length independent variants in at least one nucleotide position. Between the genetic diverse breeds, like those from Vietnam and Europe, DNA sequences between alleles differed more often. Their relevance is discussed in view of the use of microsatellite polymorphisms.

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**TOPIC: Gene-based technologies applied to livestock genetics and breeding**

# Combining gene-based methods and reproductive technologies to enhance genetic improvement of livestock in developing countries

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The advent of molecular markers allows determination of actual genotype at gene loci, without error due to random and non-random environmental effects. In the ideal situation we can directly identify genotypes at loci containing genes with substantial effects on quantitative traits (QTL). When selection is on indirect markers there is no guarantee of QTL genotype as marker alleles linked to the preferred QTL allele can be different in different families. In such a case information about linkage phase needs to be accumulated based on phenotypic and pedigree information (e.g. a progeny test). Selection based on DNA markers, either direct markers: Genotype Assisted Selection (GAS) or indirect markers: Marker Assisted Selection (MAS), is most useful for traits that are hard to measure and have low heritability. Selection of animals based on (most probable) QTL genotype will allow earlier and more accurate selection, increasing short and medium term selection response and may aid in targeting genotypes for specific production environments or markets.

Novel reproductive technologies boost reproductive rates of breeding males (through artificial insemination – AI) and of females (through multiple ovulation and embryo transfer – MOET- or harvesting of oocytes in juveniles followed by *in vitro* fertilization - JIVET). The benefit arises from increased selection intensity, as well as from increased selection accuracy (larger families) and decreased generation interval (higher reproductive rates result in optimal designs with younger breeding animals). Increased reproductive rates potentially decrease effective population size, and therefore increase inbreeding. Selection for increased merit needs to be balanced against maintenance of sufficient effective population size. Therefore, selection needs to be optimized such that contributions from selected parents are optimal not only with respect to the next generation, but to future generations as well. Extra benefit from scenarios with unlimited use of reproductive technologies are restricted by the need to maintain genetic diversity (and sufficient effective population size).

Benefits for selection based on genotypic information is potentially higher in breeding programs that use technologies to boost reproductive rates, as the value of providing information about genotype is more beneficial for early selection. Moreover, GAS would provide information about within family variation, which has extra value as response to early selection based on between family differences is limited in breeding programs where loss of genetic diversity is to be controlled. Therefore, reproductive technologies potentially might provide the 'selection space' [2] which can be exploited when using genotype information.

Under optimal selection strategies, i.e. effectively under similar inbreeding scenarios, the additional response resulting from increased reproductive performance is constrained by maintaining sufficient effective population size. However, compared with natural mating strategies, response (after 10 years of selection) could be increased significantly, e.g. by about 20% and 35% for MOET and JIVET schemes, respectively [1]. Use of GAS can increase

genetic response initially but on the longer term (10 years) the advantage is much smaller even if major gene and polygenic response are optimally balanced. The initial benefit from applying GAS is lost later on because of loss of response from under utilizing the remaining polygenic part of the genetic variance. In the so-called 'juvenile schemes' where first selection occurs before the first phenotype has been measured, response based on phenotypic selection is difficult and GAS provides significantly more benefit. In this case, GAS can easily double initial response and even after 10 years of optimal selection the superiority of GAS over non-GAS selection can be in the order of 40% for MOET and JIVET schemes and about 20% for natural mating schemes [1]. Therefore, the combined application of reproductive and gene technologies in breeding programs work synergistically. In general, however, use of genotypic information in breeding programs for within breed selection will generally have limited extra benefit, unless selection based on phenotype is difficult or advanced reproductive technologies are used.

Most breeding programs in both developed and developing countries struggle to obtain rates of genetic response that are anywhere near to what might be expected based on theoretical considerations. The discrepancy is often due to the lack of control of selection decisions, which are often inefficient and uncoordinated. Implementation of advanced genetic and reproductive technologies may therefore not be the first steps needed in genetic improvement. However, when the gains in response can be significant, they should not be avoided either. Such larger benefits from gene technologies maybe expected when exploiting variation across populations, as described below.

Rather than exploiting existing QTL in within breed selection, a more likely scenario is that valuable QTL will be introgressed into other populations. Either indigenous breeds may contains valuable QTL, but could benefit from upgrading from crossing with superior breeds, or valuable QTL could be introgressed from exogenous breeds. Examples are the Booroola gene in the Garole breed (having a moderate and desirable effect on number of lambs weaned), and a number of genes affecting resistance to endemic local diseases. Furthermore, there are many cases of QTL found in crosses of extreme breeds, and a number of those will be a candidate for introgression. Use of genotype information is likely going to be more useful in marker assisted introgression (MAI) compared with 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. Again, optimal strategies will have to consider genetic diversity as well as risk. No studies have looked at MAI scenarios in a complete framework for livestock production, but such studies would be warranted, as they would likely form an important basis for the use of genetic technologies in genetic improvement programs in developing countries.

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**TOPIC: Gene-based technologies applied to livestock genetics and breeding**

# Evaluation of the utility of the FecB gene to improve the productivity of Deccani sheep in Maharashtra, India

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The Booroola fecundity gene (*FecB*) is an autosomal gene in sheep with a large effect on ovulation rate and consequently, litter size. The Nimbkar Agricultural Research Institute (NARI) at Phaltan, Maharashtra, India (latitude 18$^{o}$N and longitude 74$^{o}$E) has embarked upon a breeding program to introgress the *FecB* gene from the Garole breed of Sunderban, West Bengal, into the local Deccani breed. Garole sheep, the probable original source of the *FecB* gene, are small-sized (average adult live weight 15 kg) and adapted to hot humid conditions. The Deccani are the native sheep of the semi-arid Deccan plateau and adult ewes weigh about 27 kg.

The reproductive performance of 188 ewes with ¼ Garole genotype (progeny of Deccani ewes mated to Garole x Deccani F1 rams), 97 of which were heterozygote carriers of the *FecB* gene (*FecB*$^{B+}$) and 91 were non-carriers (*FecB*$^{++}$), was analyzed to quantify the advantage in lamb production conferred by the gene. The percentage of abortions/stillbirths among maiden ewes was compared between ewes of the two FecB genotypes using a Z test. Other traits analysed were litter size (lambs born alive) and lambs weaned per lambing (weaning age being 120 days) as traits of the ewe. Fixed effects fitted for both traits were *FecB* genotype of ewe, birth year of ewe, year and season of lambing with age of ewe at lambing as a covariable. The interaction of ewe genotype and litter size was also fitted for lambs weaned. In addition, another model was fitted for the trait lambs weaned per lambing, with litter size instead of ewe genotype as a fixed effect. A random sire effect was fitted for both traits. A repeated measures analysis was done for both traits, using data from 1–3 lambings per ewe. Sire variance was found to be very low for both traits.

The proportion of abortions/stillbirths among heterozygote maiden ewes (0.21) was significantly higher ($P<0.05$) than that among non-carrier ewes (0.11). At the second and third parities, the proportion of abortions (0.04 and 0 respectively) was similar in both groups. Birth year of ewe, year-season of lambing and age of ewe at lambing were not significant for litter size or lambs weaned. *FecB* genotype of ewe had a highly significant ($P<0.001$) influence on litter size, as expected. The least squares mean litter size of non-carrier ewes was 1.00 and that of heterozygote ewes increased from 1.44 at the first lambing to 1.88 at the third lambing. These results are similar to those reported earlier [1] for a larger dataset including the ewes considered here.

Of the lambing ewes carrying one copy of the *FecB* gene, 54.5% , 44.4% and 25% had single lambs at the first, second and third lambings respectively (Table I). One heterozygous ewe at the second lambing and three heterozygous ewes at the third lambing had triplets. One non-

carrier ewe had twins at the first and second lambing while none had twins at the third lambing. The interaction between litter size and *FecB* genotype was significant for lambs weaned per lambing (P=0.03 at first lambing, P<0.01 at subsequent lambings). Table I below shows that lamb production of ewes bearing twin lambs is 65 to 112% higher than those bearing singles while heterozygote ewes produce 37 to 45% more weaned lambs than non-carrier ewes.

TABLE I: Least squares mean number of lambs weaned per lambing (LW) by 25% Garole ewes for the fixed effects of litter size and FecB genotype

| Fixed effect | First lambing | | | Second lambing | | | Third lambing | | |
|---|---|---|---|---|---|---|---|---|---|
| Litter size | No. of ewes | LW | S.E. | No. of ewes | LW | S.E. | No. of ewes | LW | S.E. |
| Single | 123 | 0.66 | 0.11 | 74 | 0.82 | 0.11 | 26 | 0.96 | 0.12 |
| Twin | 35 | 1.09 | 0.19 | 29 | 1.74 | 0.19 | 15 | 1.71 | 0.17 |
| Triplet | No triplets born | | | 1 | 2.99 | 0.47 | 3 | 2.87 | 0.33 |
| *FecB* status | | | | | | | | | |
| $FecB^{B+}$ | 77 | 0.90 | 0.05 | 54 | 1.33 | 0.07 | 24 | 1.45 | 0.11 |
| $FecB^{++}$ | 81 | 0.90 | 0.05 | 50 | 0.97 | 0.08 | 20 | 1.00 | 0.12 |

There are indications that the proportion of heterozygote ewes bearing twins is likely to increase substantially at the third and later parities when they reach peak production and they are therefore likely to produce significantly higher number of weaned lambs than non-carrier ewes. Lamb production performance of heterozygote ewes will be evaluated in local shepherds' flocks in the next two years.

Further reduction in the proportion of Garole genes beyond 25% is likely to yield additional benefits since Deccani sheep have been observed to have higher milk production and consequently better ability to rear lambs compared to Garole sheep. These preliminary results of performance of ewes on NARI's farm suggest that the introduction of the *FecB* gene into the Deccani under the more efficient management of native shepherds may prove successful and lead to an increase in lamb production. However, this will depend to some extent on the reproductive performance of Deccani ewes homozygous for the *FecB* gene, and this is an issue we are currently investigating.

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**TOPIC: Gene-based technologies applied to livestock genetics and breeding**

# Effect of pregnancy on sex steroid receptor mRNA endometrial expression and on prostaglandin $F_{2\alpha}$ metabolite concentrations in heifers

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The aim of this study was to investigate the effects of pregnancy on mRNA expression of estrogen and progesterone receptor (ERα, PR) in endometrial biopsy samples. It was also tested whether uterine biopsy provokes $PGF_{2\alpha}$ release and induces luteolysis or allows pregnancy to be maintained. Twenty nine heifers in heat (Day 0) were inseminated (n=21) or not inseminated (control, n=8). Blood samples for progesterone ($P_4$) determination were taken daily from Days –1 to 25. On Day 17 endometrial samples ipsilateral to the corpus luteum were taken by transcervical biopsies for solution hybridisation assay determinations of ERα and PR mRNA. In 12 heifers (4 controls and 8 inseminated) hourly bleedings were performed from 5 h before to 12 h after the biopsy to determine 15-keto-13,14-dihydro-prostaglandin $F_{2\alpha}$ (PGFM), $P_4$, and cortisol patterns by RIA. One of these heifers had already low concentrations of $P_4$ on Day 17 and was excluded. Pregnancy was determined by ultrasonography on day 35 after estrus. Data were analysed by Mixed Models analysis of SAS.

At Day 35, 6 out of 21 inseminated heifers were diagnosed as pregnant. Inseminated non-pregnant cows (n=15) were classified in two groups according to $P_4$ concentrations at Days 21-25: luteal ($P_4$> 18 nmol/L, AI non-pregnant A, n=2) or basal ($P_4$< 3 nmol/L, AI non-pregnant B, n=13), Figure 1. Heifers with luteal concentrations of $P_4$ at Day 25 (AI non-pregnant A) may have suffered early embryonic mortality and were possibly pregnant at Day 17, thus, they were considered as pregnant for mRNA analysis (pregnant, n=8).

The concentrations of ERα mRNA in pregnant heifers tend to be lower than in controls, P=0.10, but no differences were found in PR mRNA concentrations (data not shown). Recently, it has been demonstrated that pregnancy inhibits ERα mRNA expression as well as other components of the luteolytic cascade [1].

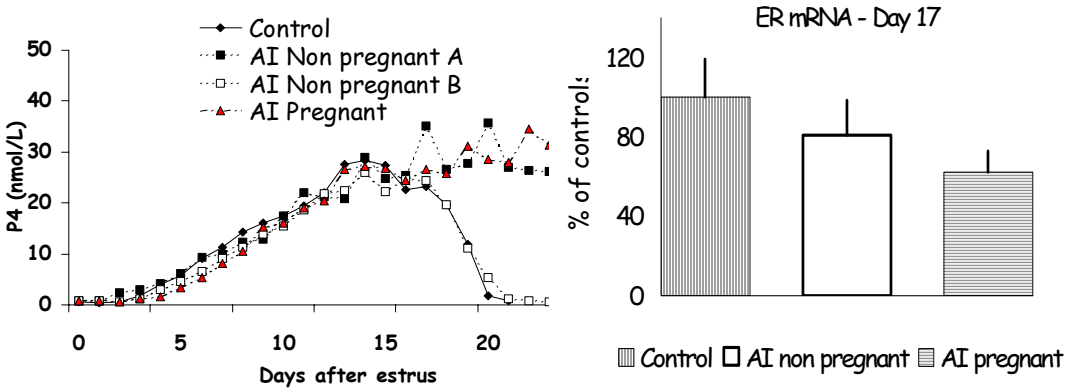


*FIG.1. Left panel: Mean concentrations of progesterone in control, pregnant, non-pregnant cows with luteal (A) or basal (B) concentrations of P4 at Day 25 postestrus. Right panel: Concentrations of mRNA of estrogen receptor α (ER mRNA) in the endometrial biopsies in control (n=8), pregnant (n=8), non-pregnant (n=13) cows on Day 17. The results are presented as percentages of control heifers.*

PGFM concentrations increased after the biopsy and remained high for the following 2 to 4 h (Figure 2, left panel). Pregnant heifers had lower concentrations of PGFM, in agreement with ER mRNA data showing that the embryo signal –interferon-τ inhibits $PGF_{2\alpha}$ secretion and the corpus luteum is maintained.

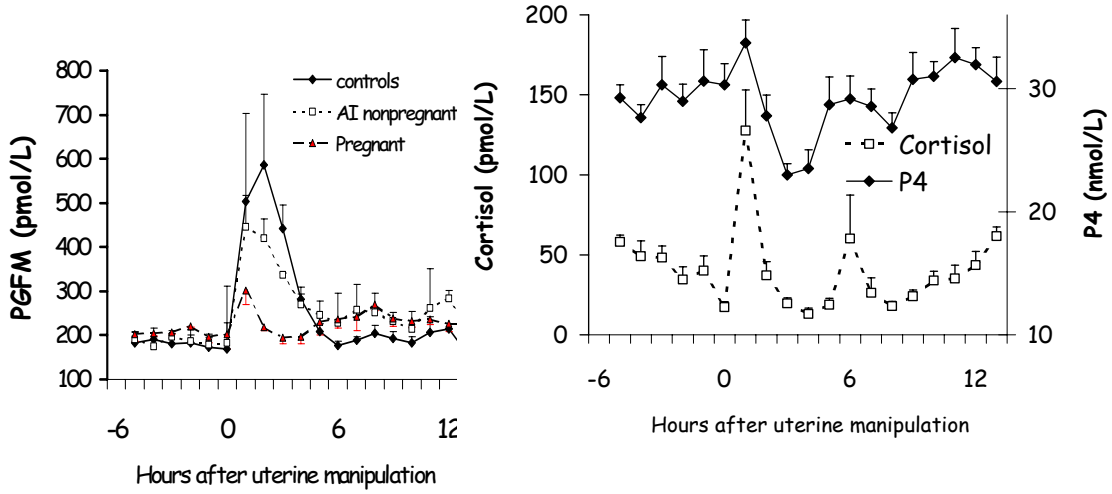


*FIG 2. Mean (± SEM) concentrations of PGFM (left panel) in controls (n=4), inseminated non-pregnant (n=4) and pregnant (n=3) heifers and progesterone and cortisol (right panel) in all (n=11) cows before and after uterine biopsy.*

Progesterone concentrations increased in the first blood sample after the biopsy, and decreased 2 to 4 h later in a temporal pattern consistent with the increase in PGFM peak, with both returning to normal soon thereafter. No statistical differences in $P_4$ or cortisol concentrations between groups were detected. The $P_4$ increase in the first bleeding after the biopsy was coincident with the cortisol peak at that moment (Figure 2), showing that the procedure provoked a stress response. Progesterone and cortisol were correlated ($r=0.21$, $P<0.01$), as reported previously [2] and may be due to the stress of the uterine biopsy since both $P_4$, a precursor to cortisol, and cortisol respond to ACTH stimulation. Uterine biopsies in

cattle may be used for clinical diagnosis of endometritis, and the measurement of plasmatic PGFM has been suggested to aid in the diagnosis of endometritis [3].

In this study, we have shown that pregnancy affects endometrial expression of ERα mRNA and plasma concentrations of PGFM. The uterine biopsy induces a temporal release in $PGF_{2\alpha}$, which is followed by a transient decrease in $P_4$ concentrations, but this procedure does not provoke luteolysis. Thus, it can be used for uterine sampling for studies on endometrial gene expression in pregnant cows.

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**TOPIC: Gene-based technologies applied to livestock genetics and breeding**

# West African cattle breeds characterizations: Review of CIRDES genetic works

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The improvement of domestical animal breeds productivity or the animal genetic diversity maintenance to allow breeders to select animals or to create new breeds in order to adapt to environmental modifications, new diseases and societies needs, requires first a detailed inventory and, secondly, a genetic characterization of domestic animal breeds. Indeed, in developing countries, the notion of breed is not clear; visual parameters are often used even if these procedures are subjective. So it is necessary to complete this phenotypic approach by a genomic one in order to contribute to an efficient characterization. At CIRDES, a regional center for subhumid livestock research and development, these studies have been conducted during the past ten years. They permitted (i) to describe the cattle phenotypic traits and their geographical localization and to highlight the breeds threatened with extinction, (ii) to determine zebu introgression level in taurine trypanotolerant cattle (iii) to identify specific alleles of different cattle breeds, and (iv) to quantify the importance of Robertson translocation in livestock production. Data collection has been realized using a bibliography study, completed by investigations in seven countries of West Africa. Blood collection has been also done for an analysis of 4 categories of genome markers (11 blood group systems, 3 blood protein loci, microsatellites and chromosomes).

According to phenotypic description and to the conceptions of autochtone human population, 13 local cattle breeds have been identified: Ndama, Kouri, the group Baoule-Somba, the group Lagoon cattle, zebu Azawak, zebu Maure, zebu Touareg, zebu Goudali, zebu Bororo, zebu White Fulani, zebu Djelli, zebu peuhl soudanien, zebu Gobra, and their crossbreds (Zebu x Ndama and Zebu x West African Shorthorns). Nine exotic breeds have been also identified: American Brahman, Gir, Girolando, Droughtmaster, Santa Gertrudis, Holstein, Montbéliarde, Jersey and Brown Swiss; and five exotic crossbreds (Holstein x Goudali, Montbéliarde x Goudali, Holstein x Azawak, Brown Swiss x Azawak and Brown Swiss x Zebu peuhl soudanien). From this initial investigation, a map of cattle distribution in each country has been realized. The areas of heavy concentration of stock and the most important breeds have been described. In addition to that, it has been revealed that Benin Pabli breed has disappeared, that Lagoon cattle and Kouri breed are threatened with extinction, that the group Somba-Baoule is subjected to an absorption by Borgou and zebu breeds. The taurine cattle proportion is decreasing, compared with the total number of cattle. But the size of Borgou population and zebu breeds is increasing considerably in the zones where trypanosomosis risk is high.

On the other hand, the analysis of genome markers polymorphism has permitted to identify specific alleles for cattle characterization and to identify the genetic reasons of some trypanotolerant cattle declining. So, some alleles were found to be significantly ($p<0.01$) correlated to breeds (107 bp of HEL1 locus and 191 bp of ETH151 locus to zebu; 197 bp of ETH152 locus to Lagoon and 139 bp of locus ETH225 to Somba). These allele frequencies in the Borgou population were roughly intermediate between those in zebu and taurine breeds. The results of statistical analysis have permitted to define the rate of zebu gene crossing in trypanotolerant cattle (21.5% in Somba cattle and 1.2% in Lagoon cattle); and to determine the degree of zebu gene introgression in Somba cattle (1.5% and 21.5% according to blood groups aspect and to hemoglobin and albumin combination). Y chromosome morphology has been found to be accrocentric in zebu cattle but submetacentric or metacentric in taurine cattle. Robertson's translocation has been observed and its prevalence has been calculated in Lagoon cattle (0.0%), in Somba cattle (10.4%) and in Borgou cattle (18.3%). These observations ought to be take into account for a regional programme development aiming at preserving and conserving the endangered breeds to maintain the biodiversity.

# PANEL DISCUSSION 1: WHICH GENE-BASED TECHNOLOGIES ARE MOST LIKELY TO SUCCEED IN ENHANCING ANIMAL PRODUCTIVITY IN DEVELOPING COUNTRIES?

**Moderator: J. Donelson, USA**

**TOPIC: Which gene-based technologies are most likely to succeed in enhancing animal productivity in developing countries?**

# Application of gene-based technologies directed at commensal gut bacteria to solve animal productivity constraints in developing countries

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Animals of a wide range of orders and classes have a portion of their digestive tract adapted to accommodate a fermentation which assists in digestion as well as providing a variety of other benefits. Advances in our understanding of fermentative digestion have tended to obscure the vital role that the gastrointestinal microbiota plays in the physiological, immunological and protective functions of the host animal. Indeed it is estimated that microbial cells outnumber host cells by a factor of 10 and that animals and humans contain 90% of their total cells as prokaryotic cells. The association of microbes with tissues of the gastrointestinal tract of animals during evolution has resulted in a balanced relationship between resident microbes and host. Numerous biochemical, physiological and immunological features that are considered intrinsic characteristics of animal species are actually responses by the animal to the physical presence and metabolic activities of the normal indigenous microbiota. This microbial challenge has modified the course of evolution in animals resulting in the selection of animal microbe relationships which are dynamic, complex and vary tremendously ranging from competition to cooperation. In fact, we can consider the gut microbial population to be the most metabolically active and rapidly renewable organ of the body.

The microbial community inhabiting the gastrointestinal tract is represented by all major groups of microbes and is characterized by high population density, wide diversity and complexity of interactions. Our current knowledge of gut microbial diversity and ecology is largely based on classical anaerobic culture techniques, phenotypic characterization of culturable isolates as well as light and electron microscopic examination. However, despite this vast amount of knowledge, microscopic and culture based enumeration and classification schemes of microbial community members have tremendous limitations. The two major problems faced by microbial ecologists studying the gut ecosystem are the inevitable bias introduced by culture-based enumeration and characterization techniques, and the lack of a phylogenetically based classification scheme. These limitations can be overcome by the application of modern molecular techniques based on sequence comparisons of nucleic acids and used to provide genotypic characterization while at the same time providing a classification scheme which predicts natural evolutionary relationships. Importantly this molecular approach does not rely on cultivation but rather the analysis of community DNA, representing all resident microbes, extracted from intestinal samples. The powerful combination of molecular biology and Woese's new phylogeny has created the now recognized field of molecular microbial ecology. This is defined as the application of molecular technology, usually based on comparative nucleic acid sequence information, to identify specific microorganisms in the gut environment, to assign functional roles to these specific microorganisms, and to assess their significance or contribution to specific metabolic

and physiological processes in the gastrointestinal tract. The advantages and limitations of various techniques employed for molecular microbial ecology studies will be reviewed in an effort to identify which gene-based technologies directed at commensal gut bacteria are most relevant and applicable to solving animal productivity constraints in developing countries. The use of these techniques has lead to major advances in our knowledge and will provide the first complete description of the gastrointestinal ecosystem.

The latest and most powerful technologies revolutionizing microbiology are based on genomics – the mapping and sequencing of genomes and analysis of gene and genome function. Genomics refers to a suite of functional and comparative methods that capitalize on the availability of entire or high coverage draft sequence. More than 100 microbial genome sequences have been completed providing information on more than 300,000 predicted genes with approximately half of these being of unknown function and potentially novel. These novel genes represent exciting new opportunities for future research and potential sources of biological resources for exploration and exploitation. The development of microarray technology is the first step in the experimental use of whole genome sequences and enables a thorough analysis of gene expression patterns in different environmental conditions. In this approach, individual DNA probes are arrayed on a small glass surface and labeled cDNA is hybridized onto the array. The amount of fluorescence at each DNA probe spot correlates with the abundance of specific mRNA transcript in the cell. This approach enables characterization of transcriptionally regulated pathways at a genomic scale. Comparison of closely related genomes can be achieved cheaply using DNA microarrays based on one completely sequenced representative. DNA microarray analyses will only provide information on which genes present in an arrayed and sequenced genome are absent from probe genomes, not which additional genes may be present in the probe. Genomic subtraction methods can provide this reciprocal information. An improved and more sensitive PCR-based method suppressive subtractive hybridization has been developed for this purpose. Finally, cloning large fragments of DNA isolated directly from microbes in the gut environment provides access to community DNA – the metagenome. Large insert Bacterial Artificial Chromosome (BAC) libraries can be used to detect gene expression from poorly studied difficult to manipulate or as yet uncultivated species. Importantly the metagenomic approach enables the linkage of specific functions with the phylogenetic group responsible for them.

**TOPIC: Which gene-based technologies are most likely to succeed in enhancing animal productivity in developing countries?**

# Animal breeding in developing countries based on gene-based selections

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Background information on the use of molecular genetic markers to detect and select for genes controlling genetic variation (quantitative trait loci, QTL) is provided by Williams and van der Werf (these proceedings). Use of QTL is predicted to be most beneficial for traits that have low heritability or are difficult, expensive or impossible to record during a breeding program. These conditions apply to disease resistance and adaptation traits in the low to medium inputs systems of the developing world and use of QTL could therefore be expected to be particularly beneficial here. The failure to use QTL information in the developing world reflects a lack of investment in QTL mapping. Such investment is needed not only to detect QTL that could be useful in genetic improvement programs, but also to design improvement programs utilising QTL information that would be sustainable under developing world conditions. A strategy for use of molecular markers that is hypothesis driven and has clear goals and routes to impact poor farmers is outlined here. The strategy is presented in greater detail by Gibson [1].

Population genetics theory predicts that natural selection will fix different genetic solutions in populations that are isolated from each other. Selection acts stochastically on the variation available, and this variation will differ in nature and extent between populations. The more genetically distinct are any two populations, the greater the likelihood they will contain distinct genetic polymorphisms and the greater the chance that selection will lead to fixation of different genetic solutions to the same problem in the two populations. Experimental support for this theory exists in model species and most recently also for the case of trypanosomosis tolerance in livestock.

While there is enormous functional diversity in the characteristics of livestock breeds, there are few cases where any breed has achieved a perfect solution to a given problem. Trypanotolerance in cattle and gastrointestinal helminth resistance in sheep are good examples, where breeds exist that are able to survive and produce under disease challenge, but such breeds still perform better in the absence of the disease. It would be desirable to produce animals with even higher resistance to disease, which would be able to thrive under the highest challenge in the absence of other disease control measures. There are well documented examples of several distinct breeds of a given species having evolved partial resistance to a given disease. A good example is gastrointestinal helminth resistance in sheep, with at least 8 breeds of sheep having been recorded as having some degree of resistance. Given the general lack of information on the characteristics of livestock breeds there are probably many more undocumented examples.

In order to identify the best possible genotype for each of a range of production environments, the ideal situation would be to test all breeds with potentially useful characteristics globally, along with all their crosses in each production environment. In practice such testing is not

feasible, due to economic and logistical limitations. It would be feasible in many cases to undertake testing of just two breeds from two different countries (or regions). Many countries would see the advantage of a reciprocal exchange of germplasm with another country, which could overcome concerns related to benefit sharing in many cases. The critical question is which two breeds would maximise the probability of being able to develop a better genotype than currently exists? Obviously choice of breeds will involve careful examination of existing data on breed performance and the environments under which they evolved. Where it is desired that a particular trait be further improved one consideration would be the likelihood that two breeds have evolved different mechanisms of adaptation, such that a higher level of adaptation (and/or performance and/or resistance) could readily be developed from a cross between them. In this case one would seek breeds that have suitable phenotypes in the targeted environment, yet are as genetically distant from each other as possible. Genetic distances among existing breeds can be estimated using molecular genetic markers and a global survey of distances among all the breeds of each species need be completed only once.

Having selected two breeds it will be important to test the hypothesis that they carry different genetic mechanisms controlling the desirable traits, before proceeding with a breeding program. A suitable method for testing that hypothesis is to perform a genome-wide interval mapping for QTL based on anonymous genetic markers in an F2 and/or backcrosses between the two breeds. Based on whether or not the hypothesis is confirmed, the size of the QTL detected and the performance of the pure breeds and the F2 or backcrosses, an informed decision can then be taken on a suitable genetic improvement program. The outcome might be to utilise one of the purebreds, or to develop a crossbreeding program, or to develop a new breed through selection from a crossbred or backcross population, or to introgress QTL from one breed to the other. In many cases the population used for testing the QTL hypothesis can also be used as the base population of a breeding program. An informed decision can be taken on whether or not the genetic improvement program should incorporate marker-based selection. This decision will depend not just on the potential value of the marker information, but also the cost and logistics of collecting and using the marker information in the genetic improvement program.

The steps outlined above, from initial mapping of global livestock diversity through to hypothesis testing and possible use of markers in selection forms a coherent strategy for detecting useful genetic variation between different populations, with a clear route to utilizing such variation using molecular genetic information.

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**TOPIC: Which gene-based technologies are most likely to succeed in enhancing animal productivity in developing countries?**

# Gene-based vaccine development for improving animal production in developing countries

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The cloning and expression of microbial genes in alternate hosts to enhance production of antigens for animal vaccines against all disease is theoretically achievable. It is essential, however, that antigens expressed in this way are known to be protective. Many years of costly research usually precedes the identification of such antigens or combinations of antigens. Thus, while conventional vaccines based on living, attenuated or inactivated microorganisms may be effective, the protective components contained in them i.e. the candidates for cloning, have yet to be found. The principal protective antigen in vaccines against foot rot of sheep and goats is fimbrial protein of *Dichelobacter nodosus*. Recombinant vaccines against this infection are ineffective if the protein subunits are not assembled and presented to the host in a manner morphologically indistinguishable from those of the natural fimbriae [1].

Availability of recombinant antigen does not necessarily avoid the need for the use of adjuvants to potentiate response. Oil emulsion vaccines, while enhancing immune response, almost inevitably cause a marked reaction at the site of injection. Livestock owners in developing countries are as likely as those elsewhere to object to these reactions. The need to find an acceptable and effective formulation adds to the cost of recombinant vaccines and their application in countries with limited resources for disease control. Another costly feature of recombinant vaccines has been the patenting of processes involving gene technology and licencing agreements for production under the protection of these patents.

In some systems antigenic competition between similar and disparate antigens limits the usefulness of even recombinant antigens that, administered individually, are highly potent [2]. In the case of programs for the control and eventual eradication of footrot in sheep and goats in Nepal this problem was overcome by the prior identification of causal serotypes and production of vaccines that were strain specific [3]. These provided duration of immunity not achievable with multivalent preparations. Wider application of this approach to vaccinology is inhibited by the requirement for rapid and accurate identification of strains of pathogens involved either in regions or in particular epidemics and the preference of commercial vaccine manufacturers for general purpose products.

The usefulness and effectiveness of vaccines based on gene technology is dependent on their inherent quality and also on the veterinary infrastructure in the countries where they are being used. This infrastructure includes knowledge of the epidemiology of target diseases and the role of vaccines in their management in the physical and social environment in different countries. Successful use of vaccines depends on having the resources to employ people to undertake vaccination and re-vaccination programs at the appropriate time or the successful completion of extension and training which together result in farmers' use of vaccines consistent with national programs. Where defined outcomes are part of national programs, assessment of response to vaccination is another component of infra-structural support. It may

also be that vaccination programs need the support of disease control legislation similar to that which exists in most parts of the developed world. Where the losses from animal disease are dramatic and obvious compliance with vaccination schemes is more readily achieved. Where losses are more insidious and related to production loss rather than deaths,veterinary service providers may need legislative support.

Cheap and effective vaccines prepared by exploiting gene technology have the potential to enhance animal production throughout the developing world. While the benefits of this technology are many it is possible that the vocal opposition, in some more developed communities, to genetically modified products will also be heard in other parts of the world. We should not assume that they are acceptable, without question, everywhere.

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## SESSION II: GENE-BASED TECHNOLOGIES APPLIED TO PATHOGENS AND HOST-PATHOGEN INTERACTIONS

**Chairperson: P.P. Pastoret, UK**

**TOPIC: Gene-based technologies applied to pathogens and host-pathogen interactions**

# Current and future developments in nucleic acid-based diagnostics

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The detection and characterization of specific nucleic acids of protozoa, rickettsia, bacteria and viruses have proven to be particularly useful for detecting pathogens of human and veterinary importance. It is also proving an invaluable tool for surveillance purposes and as a means of ensuring food security. Previous approaches towards pathogen isolation have often been tedious or even impossible. PCR, first conceived by Mullis in 1983, has proven to be a revolutionary technique for the rapid and accurate detection of numerous pathogens. The discovery and cloning of thermostable DNA polymerases has further contributed to this technology. Many additional developments, based on the basic principles of PCR, have been described e.g. RT-PCR, NASBA, RAPD, AFLP, LCR, PCR ELISA, strand displacement amplification (SDA), transcription-mediated amplification (TMA), branched DNA (bDNA), hybrid capture, immunocapture PCR. This list continues to expand with new variations on basic PCR principles.

Improvements in thermocyclers involve the development of integrated amplification and signal detection systems, including on-line real-time devices. In addition, rugged portable instruments have been designed for field use. These are particularly useful as systems for early warning in detecting biowarfare agents and outbreaks of cross-boundary and other pathogens. Fluorophores, utilising principles of fluorescence resonance energy transfer, are used as labels for probes in such real-time assays. Molecular beacon technology also utilises such mechanisms. Real-time thermocyclers allow the monitoring of amplified DNA as well as establishing sequence characteristics based on melting or hybridisation curves. Taqman chemistry makes use of such a system. Stem-loop DNA probes have been designed to have increased specificity for target recognition and include molecular beacon methodologies, suppression PCR approaches and hairpin probes in DNA microarrays. Automated sample processing or robotic devices are now also commercially available and have the advantages of greater efficiency and reduced contamination associated with conventional procedures for nucleic acid extraction.

The distinction or typing of protozoal, rickettsial, bacterial or viral strains can be done using DNA fingerprinting: including PFGE, ribotyping, genomic RFLP analysis, mitochondrial RFLP, RAPD, repetitive element-based PCR (rep-PCR) or post amplification sequencing. Sequencing of nucleic acids has proved invaluable in this regard, especially for the subtyping of viral and bacterial strains. Automated sequencers have facilitated this process and technological developments in this field can be expected to make this approach even more accessible. High-throughput DNA sequencers have played a major role in elucidating the genomes of many organisms. Pyrosequencing utilises real-time light emission during the DNA polymerisation process. Other envisaged developments include miniaturisation, thereby reducing sample size and reaction times. Prototype chip sequencers perform both PCR amplification and capillary electrophoresis. Sequencing of single molecules using

exonucleases is also now described. Further developments in the field include new dyes, polymeric matrix materials and alternative formats for capillary electrophoresis.

Reverse hybridisation uses sequence-specific linear array probes attached to nitrocellulose membranes that bind to amplicons generated by PCR. It is useful for genotyping of related organisms. Detection of single nucleotide polymorphisms, the most common stable genetic variation, is now being made using mass spectrometric applications e.g. matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF MS) on silicon chips.

Previously detection methods were frequently based on radioactively labelled molecules, now increasingly alternative approaches are being used e.g. fluorescence resonance energy transfer (FRET) as used in real-time detection devices, surface plasmon resonance (SPR) and mass spectrometry e.g. MALDI-TOF MS.

Bioinformatics has also considerable contribution to make in the design of suitable probes, analysis of sequence data, generation of phylogenetic trees and molecular epidemiological studies. The developments of improved software programmes and extensive data banks are contributing to this process. The detection of unique DNA signatures of specific organisms has been greatly facilitated by pair-ways comparisons apart from suppressive subtractive hybridization (SSH).

Microfabrication technologies are playing an important role in the construction of microchips. Contributing areas include microfluidics, materials sciences, generation of new biorecognition elements, integrated circuits. DNA microarrays with large numbers of oligonucleotide probes, allow the detection of a variety of different pathogens simultaneously. Detection technologies would include fluorescence labelling but developments in the biosensor field also have a direct application. Particularly interesting are developments in the fields of microfabrication and nanotechnology. Lab-on-a-chip devices will not only process sample materials but also perform the detection assay. Quantum dots have the potential of giving a vast repertoire of labels to molecules that would greatly facilitate the ability to distinguish between different agents. Numerous biosensor technologies utilising a variety of transducers together with biological receptors as well as nucleic acids are described. Hybridisation with target nucleic acid generates a signal detected by the transducer that can include amperometric and potentiometric electrodes, field-effect transistors, thermistors, piezoelectrical crystals, cantilevers and various optical and opto-electronical devices. The development of dip-stick or hand-held devices utilising nucleic acid detection technologies for field, patient- or crush-site use can be envisaged.

Nanotechnology promises to make many exciting contributions. More versatile labelling methods include quantum dots, gold beads and magnetic nanoparticles. Nanosensors and nanoarrays, which are a thousand fold smaller than microarrays and millions of times denser, are also likely to make significant impacts. Lab-on-a-chip devices and other integrated technologies hold particular promise in this regard. A goal for an ideal device is one capable of a broad spectrum or even universal detection of pathogens that can yield ‘yes-no’ answers. Dip-stick or hand-held devices for patient-side or on-farm use are also highly desirable.

**TOPIC: Gene-based technologies applied to pathogens and host-pathogen interactions**

# Reverse genetics with animal viruses

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Reverse genetics of negative-strand RNA viruses (NSV), which allows generation of recombinant viruses entirely from cloned cDNA, has progressed rapidly in the past decade. NSV are a large and diverse group of enveloped viruses of both medical and veterinary importance. They differ widely in morphology, genome structure and host interactions. The first NSV that was completely amenable to genetic manipulation is the neurotropathogenic rabies virus of the rhabdovirus family [1]. In subsequent years, vesicular stomatitis virus and a number of viruses belonging to the family Paramyxoviridae, including viruses causing important animal diseases such as rinderpest virus, canine distemper virus, bovine respiratory syncytial virus, bovine parainfluenza virus and Newcastle disease virus (NDV), succumbed to genetic engineering.

The ability to genetically manipulate NSV opens a wide range of possibilities to study the virus biology and develop improved vaccines. Identification and analysis of attenuating mutations using the recombinant system could lead to generation of safe vaccine strains.

Introduction of one of the previously studied mutation into an infectious rabies virus (RV) clone by replacing the arginine at position 333 of RV glycoprotein (G-protein) by an aspartic acid resulted in a dramatic attenuation. Combination of this mutation with a deletion that eliminates the interaction between RV P-protein and the cytoplasmic dynein light chain (LC8), which is presumably involved in retrograde transport of RV, further attenuates the rabies virus by 30-fold after intramuscular inoculation [2]. Since extreme attenuation may adversely affect immunogenicity, reverse genetics was used to introduce an additional G-protein to the step-wise attenuated RV to increase its effectiveness. The resultant recombinant virus may be helpful in developing a highly safe and effective live RV vaccine for oral immunizations of animals.

Reverse genetics of NSV has also helped in providing important insights into viral pathogenesis. The roles played by many accessory proteins, including V, C and NS proteins of Paramyxoviridae and influenza viruses as interferon antagonists were studied in detail using infectious clones. Since interferon antagonists are important virulence factors, their identification and modification by knocking them out or reducing their expression should provide opportunities to generate safe attenuated vaccine strains. Like other members of Paramyxovirinae, NDV produces the accessory V protein from the P gene by a process called RNA editing. Introduction of mutation into the editing site resulted in reduction of the editing frequency and as a result, V was expressed at a 20-fold lower level than the wild type NDV and was highly attenuated in chicken embryos [3]. Administration of the recombinant NDV with an editing site mutation to 18-day-old chicken embryos did not affect hatchability. Hatched chickens developed high levels of NDV specific antibodies and were fully protected against lethal challenge, demonstrating the potential use of editing-defective recombinant NDV as a safe embryo vaccine.

The ability to manipulate the genomes of animal viruses has also important implications in designing and developing marker vaccines. Different approaches can be used in designing marker vaccines. One approache that we employed for generating a marked NDV was first localizing a conserved B-cell immunodominant epitope (IDE) on the nucleoprotein (NP) gene and then successfully recovering a recombinant NDV lacking the IDE by reverse genetics [4]. In addition, a B-cell epitope of the S2 glycoprotein of murine hepatitis virus (MHV) was inserted in-frame to replace the IDE. Recombinant viruses properly expressing the introduced MHV epitope were successfully generated, demonstrating that the IDE is not only dispensable for virus replication, but can also be replaced by foreign sequences. Chickens immunised with the hybrid recombinants produced specific antibodies against the S2 glycoprotein of MHV and lacked antibodies directed against the IDE. These marked-NDV recombinants, in conjunction with a diagnostic test enable serological differentiation of vaccinated from infected animals and may be useful tools in ND eradication programs. NSV accommodates not only small epitopes, but also large foreign genes in their envelopes to be able to induce broad-spectrum immune responses. Exchange of surface proteins is also realizable with a number of NSV, demonstrating the manifold possibilities of reverse genetics in designing and developing novel vaccines.

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**TOPIC: Gene-based technologies applied to pathogens and host-pathogen interactions**

# Viral subversion of the immune system

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The continuous interactions between hosts and viruses during their coevolution have not only shaped the immune system but also the counter measures used by viruses. Studies of the last decade have described the diverse array of pathways and molecular targets used by viruses to elude immune detection and destruction. These include targeting of pathways for major histocompatibility complex restricted antigen presentation; natural killer cell recognition, apoptosis, cytokine signalling, humoral immune responses and complement activation. In this presentation, an overview of the immune-evasion mechanisms described for viruses to date, emphasizing on the importance in understanding the interaction between viruses and the immune system to improve our ability to manipulate and exploit viruses will be given.

**TOPIC: Gene-based technologies applied to pathogens and host-pathogen interactions**

# The molecular basis of livestock diseases in developing countries as illustrated by African trypanosomosis

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Viral, bacterial, protozoan and helminthic diseases of domestic livestock continue to be serious impediments to the agricultural economies of most developing countries. Many of these livestock pathogens have evolved sophisticated molecular mechanisms for evading or circumventing the mammalian immune system. These same pathogens frequently acquire resistance to drugs that are initially effective. African trypanosomes are protozoan parasites that cause the fatal diseases of ngana in cattle, surra in camels and horses and sleeping sickness in humans. They are the paradigm for a livestock pathogen in developing countries for which much is now known, yet little has been achieved in controlling or eliminating the disease.

African trypanosomes were identified as the cause of trypanosomosis more than 100 years ago and in many ways are ideal pathogens to study in the laboratory. From the perspective of research on the parasites themselves, excellent laboratory rodent models for their infection exist. They can be readily grown *in vitro* in culture flasks. Their mechanism of immune evasion is known. The completed DNA sequence of their genome is nearly determined. They can be manipulated genetically in the laboratory – genes can be mutated and deleted from, or inserted into, their genome. They contain unique organelles and metabolic pathways not found in mammals that could potentially be exploited for new drug development. They are pathogens of humans as well as livestock, so they attract the interests and enormous resources of the medical research community. Advantages also exist from the standpoint of experiments on their animal hosts. Breeds of domestic cattle that are either “trypanotolerant” or trypanosome-sensitive are well known and animals of each type have been cross-bred. The molecular basis of trypanosome-tolerance in at least one indigenous wild animal species (the Cape buffalo) has been elucidated. The reason some African trypanosome species are killed by human serum, but not by livestock serum, is understood.

Despite the extensive molecular characterizations of African trypanosomes and their interactions with livestock hosts during the past century, African trypanosomosis remains ranked among the top 10 livestock diseases impacting negatively on developing countries in Africa, Asia and South America. The many unique molecular properties of African trypanosomes will be described and discussed in the context of the other main livestock pathogens of developing countries. The reasons this information has not translated into vaccines or better drugs against trypanosomes will be presented and prospects for more successful applications of gene-based approaches against livestock diseases of developing countries in the future will be examined.