

# CURRENT STATUS AND OPTIONS FOR LIVESTOCK BIOTECHNOLOGIES IN DEVELOPING COUNTRIES

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## SUMMARY

Conventional technologies and biotechnologies have contributed immensely to increasing livestock productivity, particularly in developed countries, and can help to alleviate poverty and hunger, reduce the threats of diseases and ensure environmental sustainability in developing countries. A wide range of biotechnologies are available and have already been used in developing countries in the main animal science disciplines, i.e. animal reproduction, genetics and breeding; animal nutrition and production; and animal health.

In animal reproduction, genetics and breeding, artificial insemination (AI) has perhaps been the most widely applied animal biotechnology, particularly in combination with cryopreservation, allowing significant genetic improvement for productivity as well as the global dissemination of selected male germplasm. Complementary technologies such as monitoring reproductive hormones, oestrus synchronization and semen sexing can improve the efficiency of AI. Embryo transfer provides the same opportunities for females, albeit on a much smaller scale and at a much greater price. Molecular DNA markers can also be used for genetic improvement through marker-assisted selection (MAS) as well as to characterize and conserve animal genetic resources. Use of most molecular marker systems depends on the polymerase chain reaction (PCR), which is an important technique for amplifying specific DNA sequences. AI is practised at some level in most developing countries, primarily in dairy cattle and peri-urban areas where complementary services including milk marketing are available. The high cost of liquid nitrogen for cryopreserving semen often restricts its use far from cities. AI is usually used for crossbreeding with

imported germplasm rather than for breeding with males of local breeds due to the paucity of animal identification, recording and evaluation programmes. Lack of systems for identifying superior animals together with weak technical capacity precludes the use of more advanced technologies such as embryo transfer or MAS. Application of molecular markers has generally been limited to genetic characterization studies, usually through international cooperation.

Biotechnologies for animal nutrition and production are often based on the use of micro-organisms including those produced through recombinant DNA technology. Fermentation technologies are used to produce nutrients such as particular essential amino acids or complete proteins or to improve the digestibility of animal feeds. Microbial cultures are used to increase the quality of silage or to improve digestion, when fed as probiotics. Recombinant bacteria have been developed to produce specific enzymes and hormones that improve nutrient utilization, which can increase productivity (e.g. somatotropin) and/or decrease environmental impact (e.g. phytase). Fibre-degrading enzymes are also used to increase animal productivity and decrease environment pollutants. Although data are scarce, amino acids and enzymes appear to be the most prominent and widespread nutrition-related biotechnology products used in developing countries, and India and China have developed local industries to produce them. Various factors have limited the use of many other biotechnologies. For example, silage production is not common, thus precluding the use of microbial cultures. The uptake of recombinant somatotropin has been affected by low public acceptance, inadequate good quality feed and the low genetic potential of animals in developing countries. Fermentation of lignocellulosic materials to improve the quality of crop residues and forages has not been very effective.

Biotechnologies in animal health are used to increase the precision of disease diagnosis as well as for disease control and treatment. Monoclonal antibodies are used in immunology-based diagnostic methods including enzyme-linked immunosorbent assays. Since these methods may not allow the distinguishing of vaccinated from infected animals, molecular approaches that detect specific DNA sequences and that rely mainly on PCR are now often preferred although their use is mainly restricted to the laboratories of research institutions and larger governmental diagnostic laboratories. Vaccination is widely used as a cost-effective measure to control livestock diseases as exemplified by the soon-to-be-confirmed eradication of rinderpest. Recombinant vaccines offer potential advantages over traditional vaccines in terms of specificity, stability and safety but few recombinant vaccines are being produced commercially and their use in developing countries is negligible. The sterile insect technique is usually applied as part of an area-wide integrated pest management approach and has played a vital role in the eradication of the tsetse fly population in Zanzibar and in the control of screwworms in several countries.

### 3.1 INTRODUCTION

The challenges facing the global community in food and agriculture are enormous. According to the most recent report on the State of Food Insecurity in the World, there are now about one billion undernourished people (FAO, 2009a). Livestock contribute directly to the livelihoods of nearly one billion of the world's population. Livestock provide protein and minerals for human consumption, manure for crop production, fibre and leather for industrial uses, and draught power. Beyond their roles in providing food and inputs for agriculture and industry, livestock provide security to farmers in developing countries, especially in emergencies such as crop failures. To many of the resource-poor smallholder farmers and landless livestock keepers, animals are a living bank, facilitating both income distribution and savings. In addition, by consuming crop residues and by-products and through well-managed grazing, livestock production contributes positively to the environment, particularly in mixed crop-livestock production systems. Thus, livestock are important sources of income and employment, contributing thereby to poverty alleviation and enhancing the household food security of farmers.

Livestock production is one of the fastest growing agricultural sectors in developing countries, where it accounts for more than a third of agricultural GDP. It is projected soon to overtake crop production as the most important agricultural sector in terms of added value (FAO, 2006a). Many developing and transition countries have realized high economic growth in recent years. This, coupled with an increasing population, an expanding urban population and growth in personal incomes, is altering the lifestyle and purchasing patterns with respect to food products. Global food protein demand is shifting from plant proteins to animal proteins. Using data from 2000 as a baseline, it is projected that the demand for animal products will nearly double by 2030 and that a large proportion of this increase will be in developing countries and from monogastric animals (FAO, 2002).

This increasing demand for livestock products, termed the "Livestock Revolution", is creating opportunities for improving the welfare of millions of poor people who depend on livestock for their livelihoods and could become a key means of alleviating poverty. It has been observed that in addition to providing benefits to farmers and the animal product industry, the rapid growth in livestock production has stimulated demand for, and increased the value of, labour, land, and non-agricultural goods and services, resulting in overall economic growth. However, increasing land degradation, global warming, erosion of animal and plant genetic resources, livestock-mediated environmental pollution, severe water shortages and the threat of emerging infectious diseases pose several new challenges to sustainable animal production and food security, particularly in developing countries (FAO, 2006a; Belák and Gay, 2007; World Bank, 2009). Meeting the increasing demand for animal products, while protecting natural resources and the wider environment, is therefore one of the major challenges today.

Technological innovations have been drivers of social and economic change. They have played a pivotal role in enhancing the quality of life and the safety of animals and humans. In the last four decades there has been an unprecedented surge in the development of biotechnology in animal production and health, with gene-based biotechnologies becoming most prominent in the last decade. While the vast majority of these technologies has been developed and utilized in developed countries, they have the potential to alleviate poverty and hunger, reduce the threats of diseases and ensure environmental sustainability in developing countries. Some of the technologies have a long history of successful use, others have been used with varied success, and many more are at different stages of development and commercialization.

A number of fundamental questions can be asked about livestock biotechnologies in developing countries: To what extent are they being used today?; what are the reasons for their success (or failure)?; what emerging challenges can be addressed through their application?; what options do individual developing countries and the international community have for enabling developing countries to make informed decisions on the use of appropriate biotechnologies to enhance food security? This Chapter tries to address these critical questions.

## A. STOCKTAKING: LEARNING FROM THE PAST

### 3.2 CONVENTIONAL TECHNOLOGIES IN DEVELOPING COUNTRIES

Since the Second World War, all branches of the animal sciences – animal reproduction, genetics and breeding; animal nutrition and production; and animal health – have benefited substantially from the application of various technologies in developing countries. Although the benefits of technologies in the fields of animal genetics and breeding and animal health have produced large economic benefits – induced primarily by the adoption of artificial insemination (AI), disease diagnostics and vaccines – the role played by advances in animal nutrition should not be underestimated. Indeed, without the provision of adequate nutrition, the benefits of animal improvement programmes could not have been realized. Good nutrition is also necessary for the proper functioning of the immune system which helps keep animals healthy and productive.

The technologies used in animal nutrition have been diverse, much more so than in the other two sectors. In the early 20<sup>th</sup> century, locally available resources – mainly a mixture of crop residues, grasses and some easily available low-cost protein sources such as brans, kitchen waste and oil cakes – were used for feeding ruminants. Since the 1960s, with increased knowledge of mineral, protein and energy metabolism, concepts of balanced animal feeding emerged and several new technologies were developed. In developing countries the focus has been on enhancing the efficiency of utilizing crop residues and other roughages through urea ammoniation treatment and optimizing rumen fermentation by ameliorating nutrient deficiencies (mainly nitrogen and minerals) in low quality roughage. Approaches used included adding minerals, nitrogen in the form of non-protein nitrogen and tree leaves to roughage based diets; chopping and soaking roughages in water, which increases intake, is also being practised.

Productivity in peri-urban dairying and other commercial livestock units has been increased by using compound balanced rations of locally available ingredients; mineral mixture supplementation including the use of urea-molasses mineral blocks; the production, conservation and use of green fodder; the enrichment and densification of crop residues; the production of by-pass proteins, by-pass fat and chelated amino acids. For poultry and pigs, the nutritional provisions have shifted from the use of backyard feed resources to balanced feeding using conventional feed resources, especially on commercial farms. However, improving animal productivity has also hinged on striving for greater environmental stability. Imbalanced feeding results in the release of excess nitrogen, phosphorus and other nutrients into the environment, thereby causing pollution. Environmental pollution due to excessive feeding is particularly serious in intensively managed farms.

In the area of animal reproduction and breeding, cytogenetics has played an important role. Karyotyping technology is used to screen animals for chromosomal aberrations to assess subfertility and infertility in dairy animals. In some developing countries, open nucleus breeding systems and progeny testing programmes involving proper recording and analysis of necessary information for reliable decision making along with population and quantitative genetics have led to the development of highly productive animals when provided with the proper nutritional inputs and suitable housing and management. The basis of these systems is predicting the breeding values of the animals using phenotypic and genealogical information. Technologies such as AI and pregnancy diagnosis have been extensively used to transfer the improved germplasm to developing countries although natural mating is still the most common practice for breeding farm animals in such countries.

Since the early 20<sup>th</sup> century, the focus in animal health has been on the eradication of infectious diseases by slaughtering infected animals and in some cases, also associated animals. Recently vaccination has been used. Vaccination is the introduction (often by injection) of biological material into an individual to increase its immunity to a given disease. Its first use is attributed to Edward Jenner in the late 1700s. The biological material typically resembles the disease pathogen and prepares the immune system to react to subsequent infections. In the 1940s, the advent of antibiotics revolutionized the treatment of common diseases and these also encouraged surgical interventions. During the last decades, productivity-reducing subclinical diseases such as those caused by internal parasites have been treated with various antibiotics and drugs. For some livestock species, antimicrobials were also used as growth promoters. This last practice has not been without controversy and it is believed that misuse has contributed to drug resistance in parasites and bacteria.

The concepts and analytical techniques of epidemiology and their careful application have been a very significant factor in disease prevention in the last four decades. The availability of statistical methods, software and computing power allowed handling a large body of datasets, resulting in effective and fast decision-making and a better understanding of diseases. Epidemiology allowed for the simultaneous evaluation of the effects of various environmental, host and pathogen-related factors on disease incidence and transmission. Information on the effectiveness of vaccines under field conditions was also assessed by epidemiological methods. Other conventional techniques such as the clinical pathological analysis of specimens for the diagnosis or confirmation of diseases in farm animals and serological screening for various infectious agents have contributed significantly to monitoring and control programmes for many transboundary animal diseases. Traditional diagnostic tools such as virus neutralizing tests and virus isolation have a long history and remain the “gold standards” for serological and virological investigations. These have been invaluable

tools for diagnosis of diseases. Vaccines developed through traditional approaches have also had a major impact on the control of foot-and-mouth disease (FMD), rinderpest and other epidemic and endemic viral, mycoplasmal and bacterial diseases.

### 3.3 ANIMAL BIOTECHNOLOGIES: DEFINITIONS AND HISTORICAL PERSPECTIVE

Biotechnology has been practised since the beginning of animal husbandry. The evaluation and selection of different breeds started with the domestication of animal species around 12 000 years ago which was led by the wish to obtain traits dictated by social, nutritional and environmental needs with no understanding of the molecular processes involved. In 1919, Karl Ereky, a Hungarian engineer coined the term “biotechnology” and described it as the process by which products could be synthesized from raw materials with the aid of living organisms. In this and the other FAO background documents for ABDC-10, the definition of biotechnology follows that of the Convention on Biological Diversity (CBD), i.e. “any technological application that uses biological systems, living organisms, or derivatives thereof, to make or modify products or processes for specific use”. A brief history now follows of the biotechnologies identified for discussion in this Chapter and their definition.

#### 3.3.1 Biotechnologies in animal reproduction, genetics and breeding

The advent of AI in the 1930s represented the start of a revolution in traditional animal breeding. The subsequent discovery in the 1950s that glycerol could act as a cryoprotectant for semen removed practical barriers to the use of AI, expanding its potential exponentially. Prolonged storage of spermatozoa in a deep frozen state allows a single male to mate with thousands of females without restrictions imposed by geography and time. These developments were followed by oestrus synchronization, multiple ovulation induction and embryo transfer (ET), sperm and embryo sexing, and *in vitro* embryo production and cloning by nuclear transfer. In addition, recent developments in molecular markers coupled with the use of bioinformatics opened the possibility for identifying genomic variation and major genes for genetic improvement of livestock. The ongoing move to use molecular markers in conjunction with reproduction technologies such as AI and *in vitro* production of embryos is likely to accelerate further genetic change to obtain animals with desired traits.

*Artificial insemination:* Semen is collected from donor male animals, diluted in suitable diluents and preserved in liquid nitrogen. Fresh or frozen diluted semen is manually inseminated into the reproductive tract of an ovulating female to achieve pregnancy.

*Sperm sexing:* Depending on the species, X chromosome-bearing sperm contain 2–5 percent more DNA than sperm bearing the Y chromosome. Different sperm have distinct emission patterns when stained with a fluorescent dye and exposed to light. This difference allows the sperm to be separated by a flow cytometry machine. The sorted sperm can subsequently be used for AI to obtain offspring of the desired sex.

*Progesterone monitoring:* A highly specific antibody is used to measure the concentration of progesterone (the antigen) in blood or milk. This is particularly useful for identifying animals that are anoestrous or non-pregnant, improving the efficiency of AI. Radioactivity (radioimmunoassay – RIA) or fluorescence (enzyme-linked immunosorbent assay – ELISA) are used for quantification. The concentrations of many molecules of biological or agricultural interest can be measured using such procedures.

*Oestrus synchronization:* This is the process of bringing female animals into oestrus at a desired time by using a progesterone-releasing intravaginal device, intravaginal progesterone sponges, progesterone ear implant or prostaglandin treatment. The systematic administration of a combination of hormones such as gonadotrophins, prostaglandins, progesterone or oestradiol is also used. It assists in large-scale use of AI and can decrease the amount of labour used to monitor cattle for oestrus.

*Embryo transfer:* ET is the transfer of an embryo from one female to another. A donor animal is induced to superovulate through hormonal treatment. The ova obtained are then fertilized within the donor, the embryos develop and are then removed and implanted in a recipient animal for the remainder of the gestation period. The embryos can also be frozen for later use. Multiple ovulation and embryo transfer (MOET) increases the scope to select females – whereas AI limits selection to males – but its success depends upon the accurate identification of superior females and its application requires greater technical expertise and infrastructure than AI.

*Embryo sexing:* Heifers are preferred by the dairy industry and bulls by the beef industry. The pig industry generally prefers females due to higher quality and lower cost of production. Y chromosome probes are used for sexing the embryos. Karyotyping antibodies specific for male antigens and X-linked activity enzymes are also used for embryo sexing, but the use of Y chromosome specific probes seems to be the most reliable and practical method.

*In vitro fertilization (IVF):* Unfertilized eggs (oocytes) from ovaries of live donor animals are gathered by a technique referred to as “ovum pickup”. The oocytes are matured in an incubator and then fertilized with sperm. The resulting zygotes are incubated in the

laboratory to the blastocyst stage. The fertilized embryos can be transferred fresh or can be frozen. Sexed semen can be used to obtain embryos of the desired sex, which is more efficient and less complicated than the Y chromosome probe-based approach.

*Cryopreservation:* This refers to the storage of valuable genetic material (e.g. sperm, oocytes, embryos, somatic cells) in deep-frozen form in liquid nitrogen (-196 °C) for preservation and later use.

*Cloning:* The replication of DNA and other molecules and of genetically identical cells to produce an identical organism are all examples of cloning. Clones of entire organisms can be produced by embryo splitting or nuclear transfer including nuclei from blastomeres, somatic cells and stem cells.

*Recombinant DNA technology:* Simple changes in the DNA sequence of an organism's genome can have profound effects on its phenotype. Excision of a gene or even a single nucleotide can silence or "knock out" a gene, preventing it from being fully translated into the corresponding protein. In addition, because the DNA of all organisms is effectively the same molecule in terms of chemistry, insertion of one or more new genes into animal, plant or microbial cells is possible through various genetic tools. The microbes or animal cells hosting the transgenes become minute factories producing large quantities of the gene product. When recombinant DNA is inserted into the germ line of an animal, the result is a transgenic animal that is capable of passing the transgene on to its progeny.

*Molecular markers:* A DNA marker is an identifiable DNA fragment or sequence that can be used to detect DNA polymorphism. Molecular markers have a number of uses including estimation of population histories and genetic relationships within and between animal breeds (molecular characterization), as well as the determination of parentage. Markers that have a statistical association with a phenotypic trait can be used to select animals for the desired phenotype (MAS). Molecular markers may also be used to increase the efficiency of the introduction (introgression) of genes from one breed into another through repeated backcrossing of a recipient breed. Finally, although not an application for reproduction and breeding, DNA markers can be used to follow production streams containing particular components of interest, such as tracing animal products to their site of origin.

Different types of markers are available, including: a) restriction fragment length polymorphisms (RFLPs), in which DNA is cut with a specific nucleotide sequence using bacterial restriction enzymes yielding fragments of different lengths which are then separated on a gel; b) random amplified polymorphic DNA (RAPD) and amplified fragment length

polymorphisms (AFLPs) involving the use of restriction enzymes and the polymerase chain reaction (PCR); c) minisatellites, which are regions of DNA with polymorphisms in the number of repeated nucleotide sequences of around 25 bases in length; d) microsatellites, which are DNA repeats in tandem at each locus, the tandem repeats usually being two to five bases long; and, e) single nucleotide polymorphisms (SNPs), which are single base changes in DNA. SNPs are the basis of DNA chips which have thousands of complementary DNA fragments arranged on a small matrix and are capable of scoring large numbers of loci simultaneously. Sequence analysis of either specific DNA fragments or entire genomes can also be carried out.

### 3.3.2 Biotechnologies in animal nutrition and production

A number of products from biotechnological processes are added to animal feeds to increase the efficiency of production.

**Nutrients:** L-amino acids produced through fermentative processes are used for correcting amino acid imbalances in diets. Industrial production of amino acids using biotechnological approaches began in the middle of the last century. The biotechnological processes – fermentation and enzymatic catalysis – led to a rapid development of the market for amino acids due to the economic and ecological advantages these biotechnologies offered. Essential amino acids such as L-lysine, L-threonine, L-tryptophan, L-phenylalanine and L-cysteine are produced either using high performance mutants of *Corynebacterium glutamicum* or recombinant strains of *Escherichia coli*.

**Enzymes:** In the present context, enzymes are proteinaceous biocatalysts, generally of microbial origin, that improve feed nutrient availability by enhancing the digestibility of macromolecules and decreasing antinutritional factors. An additional advantage is a potential decrease in environmental pollutants from livestock production systems. Some examples are phytase, glucanase and xylanase. The first phytase preparation was launched in the feed market in 1991. Phytases that enter the market are produced from microbial strains that are either derived through mutation or by using recombinant DNA technology. Some of the phytase preparations authorized in the European Union (EU) are produced by recombinant strains of *Aspergillus niger*, *A. oryzae* and *Trichoderma reesei*. Other enzymes such as glucanase, amylase and xylanase, which are also products of microbial fermentation, have been used in monogastric diets for decades. For many years the use of exogenous enzymes in ruminants was discouraged because of the perception that these enzymes would be hydrolyzed quickly by rumen microbes. However, studies conducted in the 1990s showed that adding exogenous enzymes to ruminant diets also has the potential to increase productivity.

*Ionophores:* These are compounds that translocate ions across biological membranes and consequently disrupt the transmembrane ion gradient. An example is monensin, an antimicrobial compound that is produced in large amounts by *Streptomyces cinnamonensis*. In 1971, monensin was originally introduced into the poultry industry as an anticoccidial agent. Some countries have approved its use in the diets of swine and ruminant animals, particularly dairy cows and beef cattle.

*Single cell protein:* This is the microbial biomass or extracted proteins obtained from processes in which bacteria, yeasts, fungi or algae are cultivated in large quantities. It can be used as protein supplements in animal feed.

*Solid state fermentation:* A method for biological treatment of lignocellulosic materials to improve their digestibility and facilitate their enzymatic hydrolysis or to produce enzymes for various applications.

*Probiotics and prebiotics:* Probiotics are live micro-organisms which may confer health and production benefits to the host animal when administered in adequate amounts. These are usually from the *Lactobacillus* and *Bifidobacterium* families for monogastric animals, while *Aspergillus oryzae* and *Saccharomyces cerevisiae* are generally used for ruminants. Since the 1920s, foods containing probiotic microbes (*Lactobacillus acidophilus*) for human consumption have been marketed in Japan. *Lactobacillus acidophilus* use in the United States reached its peak around the middle of the 1930s and then faded. Since the late 1950s there has been steady interest in the study of probiotics for animals and humans. Prebiotics are non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and activity, or both, of specific microbial flora in the colon. Examples are inulin, fructo-oligosaccharide and resistant starch.

*Silage additives:* The nutritional quality of ensiled forages depends in part on the success of the fermentation process. Microbial inoculants and enzymes have been developed for addition into the silage at the time it is put into storage. These additives generally function by stimulating the fermentation process.

*Recombinant metabolic modifiers:* Since the 1920s, it has been known that injecting hypophyseal extracts stimulates tissue growth and milk secretion, and growth hormone was eventually identified as the primary source of this effect. During the 1990s, recombinant somatotropin produced by bacteria was licensed in various countries for the stimulation of production in dairy cows, swine and horses. Many countries have not approved its use.

### 3.3.3 Biotechnologies in animal health

Before the advent of recombinant DNA technology, the diagnosis and immunological prevention of infectious animal diseases was largely based on the use of whole pathogens or their physically resolved fractions. In many instances these crude methods were inefficient. Great improvements were obtained with the development of the ELISA, which has been the most popular diagnostic tool for animal diseases. Many ELISA systems now use recombinant antigens for detection of antibodies, which impart higher sensitivity, specificity, safety and acceptance compared with the use of whole pathogens. Additional major strides were made in pathogen detection after the discovery of PCR. Monoclonal antibodies and PCR have played an important role in the development of a number of diagnostic kits.

#### Diagnostics

*Monoclonal antibody-based diagnostics:* Monoclonal antibodies are produced by fusing two kinds of cells. One is an immune system cell that produces antibodies, the other a cancer cell. The fused cell inherits the ability to produce antibodies from the immune cell and the ability to reproduce indefinitely from the cancer cell. Kohler and Milstein were the first to develop a technique for the production of monoclonal antibodies in 1975 and were awarded the Nobel Prize in 1984. Monoclonal antibodies have a number of applications such as in diagnostic tests for animal diseases and progesterone assays for the reproductive management of livestock. Monoclonal antibodies have become common and essential tools for applying ELISA-based methodologies (e.g. antigen-capture ELISA and competitive ELISA), as well as Western blotting and immunochemistry techniques.

*Polymerase chain reaction:* PCR was developed in 1985 by Kary Mullis who received the Nobel Prize in 1993 for discovering the chemistry of this reaction. PCR increases the number of DNA molecules in a logarithmic and controlled manner. It results in the *in vitro* production of a large quantity of a desired DNA fragment from a complex mixture of heterogeneous sequences. PCR can amplify a selected region of 50 to several thousand base pairs into billions of copies. Molecular biology has been revolutionized by PCR. After amplification, the target DNA can be identified by many techniques such as gel electrophoresis or hybridization with a labelled nucleic acid (a probe). Real-time PCR, or quantitative PCR (qPCR), detects and measures the accumulation of a replicated DNA fragment during the amplification reaction. It enables quantification of the DNA and RNA (through cDNA production) present in a sample. For detection of RNA (for example the RNA of viruses), a cDNA copy of the RNA must first be made using reverse transcriptase. The cDNA then acts as the template for amplification by PCR to produce a large number of copies of cDNA. This method is called reverse transcriptase PCR (RT-PCR).

*RFLP and related DNA-based approaches:* DNA or RNA is isolated from the sample material (and if the starting material is RNA, a cDNA copy is prepared), the nucleic acid is digested with appropriate restriction enzymes into smaller pieces, and the fragments are then separated by electrophoresis to form bands for which the position is dictated by molecular weight. The pattern obtained on the gel (fingerprint) can be compared with known reference materials. This technique has been extremely useful in epidemiology, enabling comparison of isolates of a particular pathogen. This technique can also be combined with PCR to offer a much greater sensitivity for the identification of pathogens and is especially useful when the pathogen is available only in small numbers or is difficult to culture. Sequence analysis can also be conducted for more precise phenotype and genotype analysis.

### **Recombinant vaccines**

Recombinant vaccines are produced from cloned genes via recombinant DNA technologies, and can generally be assigned to one of three types: DNA vaccines, marker vaccines and virus-vectored vaccines.

*DNA vaccines:* This refers to the direct inoculation of a eukaryotic expression vector encoding antigenic protein into an animal, resulting in the *in situ* production of the encoded antigen with the host's tissue to produce an immune response. It also involves the delivery of pathogen-specific antibodies (intracellular antibodies) into the host to express antibody fragments inside the cell that can bind with and inactivate a pathogen.

*Marker vaccines:* A marker vaccine (live or inactivated vaccine) is either based on deletion mutants or on isolated antigenic proteins that enables differentiation between infected and vaccinated animals (DIVA). A DIVA vaccine is used in conjunction with a companion diagnostic test that detects antibodies against a protein that is lacking in the vaccine strain. Originally, the term DIVA was applied to gene-deleted marker vaccines but it can be applied to subunit vaccines, heterologous vaccines or some killed whole pathogen vaccines such as the highly purified FMD vaccine that is used in conjunction with non-structural protein-based serological tests. It can also be used for recombinant-based vaccines.

*Virus-vectored vaccines:* Many virus species including the vaccinia, fowlpox and canarypox viruses are used as vectors (delivery systems) for exogenous genes to deliver vaccine antigens. These viruses can accommodate large amounts of exogenous genes and infect mammalian cells, resulting in the expression of large quantities of encoded protein. An example of a virus acting both as a vector and a self-vaccine is the recombinant capripox virus expressing a peste des petits ruminants (PPR) virus antigen.

### **Sterile insect technique (SIT)**

The SIT for control of insect pests (in the present context, screwworm and tsetse flies, which cause widespread disease in livestock with enormous economic consequences for livestock keepers and governments) relies on the introduction of sterility in the females of the wild population. The sterility is produced following the mating of females with released males carrying dominant lethal mutations in their sperm that have been induced by ionizing radiation. It is an environment-friendly method of insect control and is usually applied as part of an area-wide integrated pest management (AW-IPM) approach.

#### **3.3.4 Trends**

Some clear trends were seen in this Section. Fermentation-based animal biotechnologies were developed prior to the 1950s. From 1950 to 1980, the livestock industry reaped substantial benefits from biotechnologies such as AI and oestrus synchronization. Since the 1980s, DNA-based technologies have played an increasingly important role in making animal production more efficient, economical and sustainable. Tremendous growth in molecular genetics and genomics research has taken place since the 1980s and may revolutionize the way animal genetic resources are managed and used in the future.

A common theme in the brief historical perspective presented here is that the biotechnologies have generally become progressively more complex over time, usually requiring increasingly well-trained and skilled human resources and often greater investment in laboratory infrastructure. Opportunities and risks have both tended to increase over time and approaches for analysing potential costs and benefits are becoming increasingly necessary. An important lesson that can be learned from past trends is that future biotechnologies will require an even higher degree of preparedness if their full potential is to be exploited. Biotechnology will undergo even more dramatic changes in the years to come than in the past.

## **3.4 CURRENT STATUS OF APPLICATION OF LIVESTOCK BIOTECHNOLOGIES IN DEVELOPING COUNTRIES**

Quantitative information on the current status of use of animal biotechnologies in developing countries is lacking, except the use of some assisted reproductive biotechnologies such as AI, ET and molecular markers. The generation of quantitative information on these biotechnologies was possible due to a painstaking and well organized study conducted by FAO in which information on a country's capacity to manage its animal genetic resources for food and agriculture was gathered. Reports were received by FAO from 169 countries between 2002 and 2005 and published in the State of the World's Animal Genetic Resources (FAO, 2007).

### 3.4.1 Biotechnologies in animal reproduction, genetics and breeding

#### Artificial insemination

Among this set of biotechnologies, AI is the most widely used both in developing and in developed countries. A large number of AIs are performed globally each year, more than 100 million cattle, 40 million pigs, 3.3 million sheep and 0.5 million goats (FAO, 2006b). In India alone, 34 million inseminations were carried out in 2007 (DADF, 2008). The total number of inseminations in Brazil in 2008 was 8.2 million (ASBIA, 2008). According to FAO (2007), of the 42 African countries that submitted reports, 74 percent reported using AI. This proportion was smaller for Southwest Pacific countries (55 percent) and greater for Asia (86 percent), Latin America and the Caribbean (95 percent) and the Near and Middle East (100 percent). Nearly all countries in Europe and the Caucasus region (97 percent) reported using AI and in North America the figure was 100 percent.

Of the African countries that responded, 17 percent reported using ET and 14 percent molecular genetic technologies. For Asian and Latin American and Caribbean countries the numbers were considerably greater, with 47 percent and 50 percent respectively using ET, and 86 percent and 73 percent using molecular genetic technologies. The relative use of these biotechnologies was: AI followed by ET and then molecular genetic technologies. The gap in the application of these technologies between developed and developing countries was greatest for molecular genetic technologies, followed by ET and then AI. A large number of countries in developing regions did not apply these biotechnologies routinely, and their use in small-scale or low-input systems is very limited.

In respect of Africa, Asia and Latin America and the Caribbean, the following conclusions can be drawn about AI (FAO, 2007):

- It is mostly used for cattle production systems, especially in the dairy sector. In Africa and Asia its use is concentrated in peri-urban areas. Other species for which AI is used in all three regions are sheep, goats, horses and pigs, with use more common for sheep and pigs than goats and horses. In addition to these species, AI is used in Asia for chickens, camels, buffaloes and ducks, and in Latin America and Caribbean regions for rabbits, buffaloes, donkeys, alpacas and turkeys.
- Semen for AI is mostly from exotic breeds and used in the expectation of increasing the production of local livestock populations. Semen from local breeds is also used for this purpose, but to a lesser extent. In Côte d'Ivoire, semen from trypanotolerant cattle has been used and exotic semen has also been used for crossbreeding with naturally trypanotolerant cattle.
- Most AI services are provided by the public sector but the contribution of the private sector, breeding organizations and NGOs is also substantial (Table 1).

TABLE 1

## NUMBER OF PUBLIC AND PRIVATE SECTOR ORGANIZATIONS IN AFRICA, ASIA AND LATIN AMERICA AND THE CARIBBEAN PROVIDING ARTIFICIAL INSEMINATION SERVICES

	Africa	Asia	Latin America and the Caribbean
Public sector	26	17	11
Private sector	12	6	9
Breeding organizations	2	5	5
NGOs	8	4	not reported
Universities	2	1	not reported

Countries providing information on AI service providers: Africa, 26; Asia, 17; Latin America and Caribbean, 17.

Source: adapted from FAO (2007)

- Concerns have been raised regarding the loss of biodiversity due to inappropriate and poorly planned use of AI to inseminate locally adapted cattle with imported semen for increased production.
- Most developing countries in Africa and Latin America do not have a clear breeding policy in place.

The country reports also indicate that nations such as Bhutan, the Democratic Republic of the Congo, the Gambia, Guinea and Laos wish to initiate AI activities but need to build the necessary infrastructure and capability required for initiating sustainable programmes. Cape Verde, Chad, the Cook Islands, Ghana and the Sudan all reported having started AI in the past but having stopped due to financial constraints. The AI infrastructure has subsequently deteriorated in these countries (FAO, 2006b). The availability of economically priced liquid nitrogen for the cryopreservation of semen is a particular constraint.

### Progesterone measurement

Radioimmunoassay for measuring the hormone progesterone provides information both on the problems in breeding management by farmers and on the deficiencies in the AI services provided to them by government, cooperatives or private organizations. FAO cooperates with the International Atomic Energy Agency (IAEA) in assisting countries to use nuclear techniques and related biotechnologies for developing improved strategies for sustainable agriculture through the activities of the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, based in Austria. Progesterone radioimmunoassay based on <sup>125</sup>I has been one of the cornerstones of the support provided by the Joint FAO/IAEA Division for improving the productivity of livestock in many developing

countries, and the capacity to use this technique at the field level has been built in more than 30 Asian, African and Latin American countries through several regional networks and national programmes<sup>1</sup>.

### Oestrus synchronization

The use of oestrus synchronization in developing countries is generally limited either to intensively managed farms that are under the supervision of government livestock development departments, or to smaller farms with links to farmers' associations and cooperatives where AI is routinely used. Protocols for oestrus synchronization often include the administration of oestradiol which has been banned in the EU since 2006. This ban has implications for developing countries exporting, or aspiring to export, meat into the EU. Alternative options for synchronization do exist and these have been reviewed by Lane, Austin and Crowe (2008). However, amongst the various options available, oestrogenic compounds seem to be the most efficient and cost effective. Since the benefits of using oestrus synchronization will vary depending upon the production system, the potential benefits have to be weighed against the cost before specific recommendations can be made regarding its use.

### Embryo transfer

An evaluation of country reports (FAO, 2007) shows that only five of the African countries providing information (Côte d'Ivoire, Kenya, Madagascar, Zambia and Zimbabwe) use ET technology, all on a very limited scale. The use of ET has also been independently reported in South Africa (Greyling *et al.*, 2002). Eight out of the 17 Asian countries that provided information on the issue reported some use of ET technology, but this was largely confined to research stations. However, the demand for establishing this technology was highlighted by many countries. The animal species in which the technology has been applied are cattle, buffaloes, horses and goats. In the Latin America and the Caribbean region, ET is increasingly being used by commercial livestock producers. Twelve out of the 14 Latin America and the Caribbean countries that provided information mention the use of this technology. All reported its use with cattle, two with goats, three with horses, two with sheep, one with llamas, one with alpacas and one with donkeys. Exotic embryos were used for cattle and the dairy sector was the main beneficiary. Private sector organizations are involved in providing ET in Brazil and Chile (FAO, 2007).

Each year, the Data Retrieval Committee of the International Embryo Transfer Society provides a summary of worldwide statistics of ET in farm animals. Table 2 summarizes these figures for cattle in 2007 (Thibier, 2008), and shows that about 820 000 embryos were transferred, of which 70 percent were produced *in vivo* and 30 percent *in vitro*.

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<sup>1</sup> [www-naweb.iaea.org/nafa/aph/index.html](http://www-naweb.iaea.org/nafa/aph/index.html)

TABLE 2

NUMBER OF *IN VITRO* PRODUCED AND *IN VIVO* PRODUCED BOVINE EMBRYOS TRANSFERRED IN 2007

Region	<i>in vitro</i>	<i>in vivo</i>	Total
Africa	no data	7 416	7 416
Asia	32 462	95 733	128 195
North America	9 252	301 982	311 234
South America	195 920	66 908	262 828
Europe	5 832	97 967	103 799
Oceania	1 791	7 871	9 662
Total	245 257	577 877	823 134

Source: Thibier (2008)

About 1 percent of the total was from Africa, mostly from South Africa. About 32 percent were from South America, dominated by Brazil which was responsible for almost 30 percent of all embryo transfers worldwide in 2007. Among developing countries, a large number of embryos were also transferred in China and Argentina. While the majority of embryo transfers carried out worldwide are in cattle, Thibier (2008) also reported on ET use in other species, showing that South Africa is an important player for ET in small ruminants and that the three main countries involved in equine ET are Argentina, Brazil and the United States.

Alarcon and Galina (2009) reported that government organizations in Mexico have initiated programmes to popularize ET, particularly in small-scale enterprises not bigger than 50 cows per unit. However, based on their analysis which considered the costs of preparing the donor and recipient, embryo recovery and the resulting gestation, ET is not profitable enough for farmers to sustain such programmes on their own. These programmes had a high degree of acceptability only when the organizations provided substantial subsidies since once the subsidized programmes stopped, ET was no longer sustainable.

### Semen and embryo sexing

Although these biotechnologies do not dramatically increase the rate of genetic gain, they can increase production efficiency. At a research level, they are being developed and refined in a number of research institutions in developing countries. The involvement of private companies providing these services is likely to increase their accessibility in developing countries where AI is already established. With few exceptions, they are not widely used by breeders or farmers in developing countries (FAO, 2007). Sexed sperm is commercially available in several developing countries, including Argentina, Brazil and China (Garner, 2006; Rath, 2008).

## Cryopreservation

A large number of livestock breeds (>20 percent) are at risk of extinction (FAO, 2007). Semen and embryo cryopreservation have been used for conserving rare livestock breeds (Long, 2008). An evaluation of country reports indicates that over one third of countries use *in vitro* conservation (FAO, 2007). For example, the figure is 50 percent in Asian countries, although the state of *in vitro* conservation at the national level is very variable. Well established genebanks exist in Japan and India, and genebanks are under establishment in China, the Republic of Korea and Vietnam. Semen is preserved from all the main species, and embryos from cattle, sheep and goats are also stored. In a few countries, tissue DNA is collected from all the main species. Governments undertake these *in vitro* activities in collaboration with industry. In some other countries there is limited storage of semen at AI stations, while elsewhere, particularly in the western part of the region, no *in vitro* activities exist (FAO, 2007).

Cryopreservation of gametes, embryos, DNA or cells (for example skin fibroblasts) is a cost-effective approach for the conservation of endangered species, although using DNA or non-germ cells to regenerate an extinct breed is still problematic with available technologies. It has been suggested (Hodges, 2005) that cryopreserved cells of each breed should be stored long-term in secure locations and accessed if and when the need arises in the future, either to sequence their DNA to understand genetic differences among breeds or to use the cells in cloning to regenerate extinct breeds. Conservation of indigenous genetic resources is one of the top priorities of developing countries and several country reports noted the potential use of AI and ET for cryoconservation purposes (FAO, 2007). Due to changes induced by global warming, it is plausible that the need in developed countries for the indigenous genetic resources in developing countries will increase. This highlights the need for North-South cooperation in this area and for greater financial contributions and technical support from developed countries.

## Cloning

Since the birth of Dolly in 1996, cloning has been achieved for various species. Up to 2004, about 1 500 calves had been produced through somatic cell nuclear transfer (SCNT), mainly in Europe, North America, Japan and New Zealand, but also in South America and Asia (Heyman, 2005). China produced the first cloned buffalo in 2004 and India followed suit in February 2009. At present the production of cloned animals is at the experimental stage in most developing countries. From a research standpoint, cloning makes possible the efficient evaluation of genotype x environment interactions. At the farm level, it has the advantage of increasing the rate of dissemination of tested superior genotypes in commercial populations and possibly also of increasing the uniformity of a given livestock product for

market. A chapter in the World Organisation for Animal Health (OIE) Terrestrial Animal Health Code is dedicated to SCNT in production livestock and horses, aiming to provide a scientific basis and recommendations on animal health and welfare risks to animals involved in SCNT cloning compared with other assisted reproductive technologies<sup>2</sup>.

### Transgenesis

Although at present no transgenic livestock have been commercialized for food production, a number of transgenic animals producing therapeutic proteins in milk are at different stages of commercial development. These proteins include lactoferrin, fibrinogen and a malaria vaccine (see Table 2 in Niemann and Kues, 2007). In 2006, the European Medicines Agency approved the commercialization of the first recombinant protein (antithrombin III, ATryn) produced in milk of transgenic animals (goats). The United States Food and Drug Administration (FDA) approved ATryn in 2009. It is being used for the prophylactic treatment of patients with congenital antithrombin deficiency. A number of other transgenic farm animals have been produced but not yet commercialized, including: 1) phytase transgenic pigs which enable the better use of phytate-phosphorus and decrease manure-based environmental pollution; 2) cows that express a lysostaphin gene construct in the mammary gland to increase resistance to mastitis; and 3) pigs containing a desaturase gene derived from spinach that makes pork better for human consumption by increasing the ratio of polyunsaturated to saturated fatty acids in muscle (Karatzas, 2003; Nieman and Kues, 2007). The first approvals for transgenic animals have been for biomedical applications but it is likely that food and/or environmental applications will increase over time.

According to a survey conducted by the OIE in 2005 (MacKenzie, 2005) in which 91 countries participated (60 percent from developing countries), 4 percent of the respondents in Africa and 23 percent of the respondents in Asia reported having cloning capabilities. For transgenesis, the corresponding numbers were 8 percent and 23 percent. No Near Eastern country claimed cloning or transgenesis capability at the time of the report, but in the intervening period camels have been successfully cloned in Dubai and sheep and goats in Iran. In Europe, 18 percent and 26 percent of countries claimed cloning and transgenesis capability respectively. Asian countries lag only slightly behind Europe in their capability to produce transgenic animals.

### Molecular markers

According to FAO (2007), four countries in Africa (Cameroon, Chad, Nigeria, and Togo) reported using molecular markers to characterize genetic resources. In addition, molecular characterization of livestock has been undertaken in South Africa and in other countries

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<sup>2</sup> [www.oie.int/eng/normes/mcode/en\\_sommaire.htm](http://www.oie.int/eng/normes/mcode/en_sommaire.htm)

through international collaboration. In Asia, out of eight countries using molecular markers, six use them for genetic characterization and for the evaluation of diversity and two for MAS. The species involved are cattle, sheep, goats, pigs, buffaloes, horses, camel, deer, chicken, ducks, quails and guinea fowl. In Latin America and the Caribbean, 11 countries use molecular markers, largely for the molecular characterization of breeds: cattle, sheep, pigs, chickens, horses, goats, buffaloes and camelid species including llamas and alpacas.

Molecular marker information has not yet been widely integrated into breeding programmes in developing countries. MAS can accelerate the rate of genetic progress by enhancing the accuracy of selection and by reducing the time to gather the data needed for selection. The benefit is greatest for traits with low heritability and which are unavailable before sexual maturity or without sacrificing the animal. However, in the low-input systems existing in many developing countries it may be more difficult to realize the full value of marker information because the phenotypic and pedigree information necessary to determine associations between traits and markers is often not available.

Much of the work in developing countries using molecular markers for characterization involves international collaboration. FAO activities in the area of animal genetic resources are being complemented by programmes on molecular marker-based characterization of genetic resources in Asia and Africa by the Joint FAO/IAEA Division and the International Livestock Research Institute (ILRI). Bangladesh, China, Indonesia, Iran, Pakistan, Sri Lanka and Vietnam are participating and the focus is on building capacity to genetically characterize their breeds of small ruminants<sup>3</sup>. ILRI's programmes focus on the characterization of local poultry in Cambodia, Laos, Vietnam, Egypt, Ethiopia, Kenya and Uganda and on small ruminants from seven countries. At ILRI, work is also underway on marker identification for trypanotolerance. The identification and subsequent use of markers for trypanotolerance and helminth resistance would enhance future prospects of breeding for such traits in developing countries. The International Bovine HapMap project (Gibbs *et al.*, 2009) included two African breeds considered to be resistant to trypanosomosis. Opportunities to increase disease resistance seem particularly promising but uptake in developing countries is likely to be achieved only in the medium to long term rather than in the near future.

Marker/gene-assisted selection has been applied in the Awassi and Assaf dairy breeds in Israel for the introgression of the Booroola gene (FecB gene) for enhancing prolificacy (Gootwine *et al.*, 2003), and in India it has also been used to introgress the Booroola gene in the Deccani breed of sheep, a meat-producing breed (see Case Study 3.6.1 later). In developing countries, genotype information is expected to be initially more useful in marker/gene-assisted introgression rather than in selection within breeds (Perera and Makkar, 2005).

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<sup>3</sup> [www-naweb.iaea.org/nafa/aph/crp/aph-livestock-phase1.html](http://www-naweb.iaea.org/nafa/aph/crp/aph-livestock-phase1.html)

The recent development of DNA chips that can simultaneously type tens of thousands of SNPs has opened up the possibilities of “genomic selection” (Meuwissen, Hayes and Goddard, 2001). This approach is already being used for commercial species in developed countries and may potentially be a useful option in some developing countries. However, because few genetic analysis programmes currently exist in developing countries to provide the data needed to underpin any type of MAS, the capability for genomic analyses for the short to intermediate term will remain centred in developed countries.

### 3.4.2 Biotechnologies in animal nutrition and production

#### Nutrients and feed additives

Of the biotechnologies available to improve animal nutrition, the use of feed additives such as amino acids and enzymes appears to be most prominent and widespread in developing countries. The use of these technologies has already realized substantial economic and environmental gains. In developing countries the greatest use is in pig and poultry production, where over the last decade intensification has increased, further accelerating the demand for feed additives.

#### Amino acids

The amino acids in feed, L-lysine, L-threonine, L-tryptophan and DL-methionine constitute the largest share (56 percent) of the total amino acid market, which amounted to around US\$4.5 billion in 2004. Amino acids are mostly produced by microbial fermentation and in the world market for fermentation products, after ethanol and antibiotics, amino acids are the most important category and demand for them is increasing rapidly (Leuchtenberger, Huthmacher and Drauz, 2005). Most grain-based livestock feeds are deficient in essential amino acids such as lysine, methionine and tryptophan and for high producing monogastric animals (pigs and poultry) these amino acids are added to diets to increase productivity. Balancing of diets using amino acids also decreases excretion of nitrogen from the animals into the environment. Lysine is the first limiting amino acid for pigs and, after methionine, it is the second limiting amino acid for poultry. In 2005, the estimated demand for lysine as lysineHCl was 850 000 tons while for L-threonine (the second limiting amino acid for pigs) and L-tryptophan (third limiting amino acid for pigs) it was 70 000 tons and 3 000 tons respectively. Whereas fermentation methods for producing lysine, threonine and tryptophan are well established, cost-effective production of L-methionine has not yet been successful (Leuchtenberger, Huthmacher and Drauz, 2005). The production of methionine has been through a synthetic process or through the use of enzymes obtained from microbes. L-cysteine, generally needed for feeding to wool-producing animals, is also produced by enzymatic processes. Rumen-protected methionine and its analogues

and amino acid chelates (for increasing mineral absorption) are also used in developed countries and to a very limited extent in intensive livestock production systems in some developing countries.

### Enzymes

The use of phytase in pig and poultry feeds in intensive production systems in developing countries is significant. Phytase addition can reduce phosphorus excretion by up to 50 percent, contributing significantly to environmental protection. It also increases profitability (phosphorus resources are limited and expensive) by decreasing the amount of phosphorus added to the diet and increasing productivity by improving the availability of minerals, trace elements and nutrients for the animal. In 2007, animal feed enzymes had a market of US\$280 million worldwide, with phytase making the largest contribution. The animal feed enzyme sector grew at a rate of 4 percent per year between 2004 and 2009 and it is expected to grow annually by 6 percent from 2007 to 2012 (Thakore, 2008). The phytase market in China amounts to 5 500 tons per year.

At present, there are over 100 companies producing feed enzymes in China (Yu, Wang and Zhang, 2008). According to the China Fermentation Industry Association, feed enzyme production was 10 000 tons in 2001, forming 3 percent of China's enzyme production and 4 percent of its feed additive production (Deng, Chen and Deng, 2008). In India, the use of phytase in monogastric diets is approximately 500 tons/year (CLFMA, 2007). Other exogenous enzymes such as xylanases, glucanases, proteases and amylases and their mixtures are also added to the diets of monogastric animals in commercial farms in some developing countries. In India, 625 tons of these enzymes were used in monogastric diets in 2007 (CLFMA, 2007). Their use in developed countries is widespread. They improve digestion, remove antinutritional factors and improve productivity. The use of cellulases and xylanases has the added advantages of increasing digestibility, thereby reducing the amount of manure and possibly methane emissions from ruminants. However, the response to the addition of enzymes in ruminants appears to be variable (Rode *et al.*, 2001). The reasons for this variability are not yet fully understood. Due to a ban on the use of growth promoters in animal diets in the EU since 2006 and increasing pressure for a ban in North America, new agents for promoting growth are being investigated. The potential use of enzymes such as cellulases, xylanases and other fibre-degrading enzymes in ruminant diets is likely to increase both in developing and developed countries provided a consistent and large response is achieved and their cost is low.

### Ionophores

The use of monensin is banned in the EU, although it is used in some industrialized countries. In China, monensin can only be used as an anti-coccidian for chicken and as a growth promoting additive for beef cattle, whereas it is prohibited for use during lactation in dairy cows and laying chickens (MOA, 2001).

## Single cell protein

From the 1970s to the 1990s extensive research was conducted on single cell proteins. With the exception of some algae, however, they are not being incorporated in livestock diets in either developing or developed countries. Algae such as azolla and lemna are used to a limited extent as feed for pigs by small-scale farmers in Vietnam and Colombia.

## Solid-state fermentation

The degradation of wheat and rice straws and other lignocellulosic materials using white rot fungi that degrade lignin was also extensively researched from the 1970s to the 1990s. In general, however, the nutrient availability from the treated material is decreased due to the consumption of carbohydrates present in the lignocellulosic materials by the fungi for their growth and metabolism. The nitrogen content of the treated material is higher but a large proportion of this nitrogen is contributed by nucleotides which do not increase productivity. Probably for these reasons, this technology has never got off the ground but solid-state fermentation for producing enzymes, especially phytase for animal feeding is being employed commercially (Vats and Banerjee, 2004).

## Probiotics and prebiotics

Although probiotic and prebiotic products have been claimed to elicit several beneficial effects in both monogastric and ruminant animals, the results have been variable (Krehbiel *et al.*, 2003; Patterson, 2005). Much remains to be established about the diet, the environment, husbandry condition and dose-dependence of their effects. Despite the inconsistent results, probiotics are in use in a number of developing countries, with their use being greater for monogastrics. For example, in China there are currently more than 400 companies producing feed microbe additives, some engaged in large-scale production. Fifteen microbes have been approved for use as feed additives in China. In India, 2 000 tons of probiotics have been used in monogastric diets and the total market value of probiotics and enzymes in India is around \$US1 million (CLFMA, 2007). In Indonesia, a number of undefined probiotics for animal feeding are available on the market (H.P.S. Makkar, personal communication), but information is lacking about the number of viable microbes per unit weight or volume, their stability through processing and digestion, shelf-life and efficacy. Live microbes such as *Aspergillus oryzae* and *Saccharomyces cerevisiae* are being used increasingly in ruminant diets to improve rumen efficiency, especially in intensive production systems. A number of commercial products are available. Their use in the reduction of methane output from ruminants is also being investigated.

A success story in the use of live microbes for ruminants is the introduction of a bacterium *Synergistes jonesii* into the rumen. It prevents mimosine toxicity and enables the safe use of *Leucaena leucocephala* as a protein-rich feed in many developing countries. Manipulation of

probiotics and rumen microbes through transgenic processes to obtain microbes capable of degrading toxins holds promise (an example being genetically modified [GM] *Butyrivibrio fibrisolvens* capable of degrading a toxin, fluoroacetate); but may face obstacles for regulatory approval and adoption because of their possible adverse ecological effects.

Prebiotics are commonly fed to weanling pigs in Japan and are increasingly being used in Europe (Ficklinger, van Loo and Fahey Jr., 2003), while their use in North America is just beginning. Due to lack of information about their efficacy, the commercial use of prebiotics is not as widespread as of probiotics, and in both developed and developing countries use is limited to some research stations. Novel products in the form of synbiotics, a mix of pre- and pro-biotics are expected to be available, once more is known.

### Silage additives

The use of bacteria such as *Lactobacillus plantarum*, *L. buchneri*, *L. acidophilus*, *Streptococcus bovis*, *Pediococcus pentosaceus*, *P. acidilacti*, and *Enterococcus faecium* and yeasts such as *Saccharomyces cerevisiae* alone or their mixtures, and the use of enzymes (cellulases, hemicellulase, amylase etc.) alone or as a mix with microbial inoculants in silage production is restricted to few intensively managed commercial dairy and beef production farms in developing countries. However, the extent of their use in developed countries is higher.

### Recombinant metabolic modifiers

The beneficial effects of recombinant somatotropin in most farm animals are well established. Recombinant somatotropin technology is considered to be very effective for pigs, less so for ruminants and mostly ineffective for chickens. Recombinant bovine somatotropin (rBST) increases feed conversion efficiency and milk yield and decreases milk fat. The increase in milk yield has been reported to be about 10–15 percent, both in developed and developing countries (Chauvet and Ochoa, 1996; Forge, 1999). Administration of rBST to lactating Holstein cows also improved milk yield during heat stress without compromising fertility (Jousan *et al.*, 2007). The commercial use of rBST is common in approximately 20 countries, including developing countries, for example Brazil, Colombia, Costa Rica, Egypt, Honduras, Jamaica, Kenya, Mexico, Namibia, Peru, South Africa, Turkey and Zimbabwe (Forge, 1999; Cowan and Becker, 2006). It is banned in the EU and most other industrialized countries with the exception of the United States, mainly because of animal welfare concerns. Recombinant porcine somatotropin is permitted for use in approximately 14 countries. It increases muscle growth, reduces body fat and improves carcass composition, which gives higher market value to the product.

A prerequisite to realizing the benefits of recombinant somatotropin is feeding a good quality diet. In most developing countries, animals, particularly those raised by smallholder farmers, do not have access to such diets. In addition, the genetic potential of these animals

for production is usually low compared with animals in developed countries, giving lower “absolute” response to the administration of somatotropin, and thus decreasing the benefit to cost ratio. Therefore, the use of recombinant somatotropin in developing countries could be expected to be commercially viable only in intensive livestock production systems. However, before adopting this technology, an economic analysis of the production unit should be available. Regular administration of recombinant somatotropin could also become a constraint under some production conditions. The risks of increasing mastitis or latent viral or other pathogenic infections (the elimination of xenobiotics is slower in animals receiving rBST) and the negative effects of rBST on fecundity and fertility when administered before breeding must also be taken into consideration before introducing this technology (Chilliard *et al.*, 2001).

### Genetically improved feed

While crops are covered separately in Chapter 1, it should also be mentioned that the genetic enhancement of feed crops represents another important pathway towards the improvement of animal nutrition. A range of conventional strategies and biotechnology tools have been used for this purpose. For example, in the 1960s, scientists discovered that maize with the opaque-2 gene had higher levels of lysine and tryptophan which are essential amino acids for monogastric animals. This led to the release of “quality protein maize” (QPM) varieties developed through conventional or marker-assisted selection. Over 1.2 million ha have been planted to QPM varieties and hybrids in developing countries which are used for direct human consumption or as animal feed (Vivek *et al.*, 2008). Although no GM crops specifically developed for animal nutrition purposes have yet been commercially released, they are in the pipeline. For example, it is estimated that GM maize containing the gene encoding the phytase enzyme may be commercialized in China in 2010 (Stein and Rodríguez-Cerezo, 2009). Further details on applications of crop biotechnologies in developing countries are given in Chapter 1.

### Molecular gut microbiology and rumen micro genomics

Although at the research stage, these approaches have high potential for increasing livestock productivity by providing a better insight into the digestive physiology of livestock. Since the development of the Hungate tube in 1950, understanding has increased about the role of strict anaerobic rumen micro-organisms in the digestion of feed, the microbiological transformations that occur in the rumen, and the physiological importance of the products released from feed as a result of microbial digestion. The molecular era in the field of rumen microbiology started with the building of gene libraries, cloning and manipulation. By the early 1990s there were over 100 cellulase genes sequenced from rumen bacteria. Cellulolytic

bacteria were found to contain multiple copies of genes from a variety of cellulase gene families, and in some cases the cellulases were assembled into cellulolytic complexes called cellulosomes. From the complexity of the genetic system required to degrade cellulose it became obvious that it would be very difficult in the short term to make a significant impact on cellulose hydrolysis using genetic manipulation. At present, another technical challenge is to introduce and maintain recombinant strains in the mixed rumen population, and survival of new strains is not well understood.

Stahl *et al.* (1988) described the use of 16S rRNA gene sequences to classify and identify rumen microbes based on DNA sequence. This study and the development of PCR revolutionized the study of diversity and complexity of ruminal microbial communities without the need to culture them. The ongoing “omics” phase in rumen microbiology is giving functional dimension to the changes in microbial ecology of the rumen and is likely to provide opportunities for manipulation of rumen microbes for enhancing the efficiency of fibre utilization, decreasing methane production and increasing the utilization of feeds containing toxins and antinutritional factors.

So far, the direct benefit of these advances to developing countries has been by providing a means to track the establishment of a bacterium, *Synergistes jonesii* (which degrades mimosine, a toxic component) in the rumen by using a PCR-based technique, enabling better utilization of *Leucaena leucocephala* leaves as livestock feed. PCR-based tracking techniques would also be useful in developing effective probiotics for monogastric and ruminant animals. In developing countries, PCR-based detection methodologies are better developed for animal health applications. A strategic collaboration between the health and production scientists within developing countries would certainly make animal nutritionists better able to address challenges in economic animal nutrition. The Joint FAO/IAEA Division has helped to build capabilities in Brazil, China, Colombia, Cuba, Ethiopia, India, Thailand and Turkey to evaluate microbial diversity, quantify microbes without culturing them, and study changes in commensal microbes as affected by additives and feeding strategies<sup>4</sup>. These PCR-based methodologies will complement conventional feed evaluation methodologies to develop rational feeding strategies in developing countries.

### 3.4.3 Biotechnologies in animal health

In vast areas of the world, animal diseases cause severe losses in livestock systems, wildlife and, in the case of zoonotic diseases, humans. Often the devastation of acute diseases which kill a high percentage of animals or the long-term effect of chronic diseases, has a massive effect on economies and hence on the overall conditions for human existence. Recent

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<sup>4</sup> [www-naweb.iaea.org/nafa/aph/crp/aph-molecular-techniques.html](http://www-naweb.iaea.org/nafa/aph/crp/aph-molecular-techniques.html)

incidences of emerging and re-emerging transboundary animal diseases have resulted in huge economic losses. Since 2005, the OIE has reported the occurrence of FMD in Africa, Asia and South America; classical swine fever (CSF) in Africa, Asia and Europe; and highly pathogenic avian influenza (HPAI) in Africa, Asia and Europe. The Secretariat of the Global Framework for the Progressive Control of Transboundary Animal Diseases (GF-TADs), a joint FAO/OIE initiative, carried out regional consultations to identify priority diseases and the best ways for their administration, prevention and control. From this, it was noted that FMD was the first global priority (Domenech *et al.*, 2006). Rift Valley fever and HPAI were ranked as major zoonotic diseases. PPR, contagious bovine pleuropneumonia. African swine fever and CSF were also regionally recognized as top priorities (Domenech *et al.*, 2006). The specific detection of agents causing such diseases and establishment of early warning systems are major tasks since timely action could prevent their spread to large animal populations and many countries.

The widespread occurrence of animal diseases in developing countries is one of the major factors responsible for decreasing livestock productivity in these countries. Generally, these diseases mostly affect resource-poor livestock farmers and hence their effective control is essential for poverty alleviation. Vaccination and molecular-based diagnostics are increasingly being used to improve control strategies. The application of inactivated or live attenuated vaccines offers a cost-effective measure to control or even eradicate an infectious disease as exemplified by the near-eradication of rinderpest. During the last two decades these vaccines have played a more prominent role in enhancing livestock production in developing countries. In 2003, the estimated market value of animal diagnostics was around US\$0.5 billion while that of animal therapeutics such as vaccines, pharmaceuticals and feed additives was US\$15.1 billion (Elder, 2004). The number of licenced animal products is 105, most of them biological, including veterinary vaccines and diagnostic kits. The animal health industry invests over US\$400 million annually in research and development (R&D) and the value of animal health biotechnology-based products is US\$2.8 billion (Belák and Gay, 2007), while the contribution of veterinary vaccines to this global market is approximately 23 percent (Meeusen *et al.*, 2007).

## Diagnostics

Molecular-based serological techniques, for example those using monoclonal antibodies and recombinant antigens in ELISA, as well as PCR-based diagnostics, are widely used in developing countries. Information on their application for specific diseases, as well as detailed descriptions of the methods involved, are provided by the OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (OIE, 2008), whose objective is to provide internationally agreed diagnostic laboratory methods and requirements for the

production and control of vaccines and other biological products. It covers standards for diagnostic tests and vaccines for the large number of diseases listed in the OIE Terrestrial Animal Health Code.

Methods based on ELISA and PCR are in wide use globally. In Africa, ILRI and the ARC-Onderstepoort Veterinary Institute, South Africa, are leaders in this area. Currently, ELISA forms the large majority of prescribed tests for the OIE-notifiable animal diseases, with many kits available in developing countries. Nevertheless, despite a great deal of technology transfer many countries still lack the capacity to exploit the full potential of this type of assay to develop tests, and their level of training needs to be improved. The OIE has developed a twinning programme between OIE Reference Laboratories and candidate laboratories for scientific capacity building and the improvement of expertise within developing countries (OIE, 2006). National laboratories such as the Laboratoire National de l'Élevage et de Recherches Vétérinaires in Senegal, the National Veterinary Institute in Ethiopia and the Central Veterinary Laboratory in Niamey, the Niger are examples of institutions that possess good diagnostic capability. In South Africa, efforts are being made to develop molecular diagnostic kits for tick-borne diseases. In Asia and Latin America, public sector production of diagnostic kits for animal diseases can be found in China, India, Thailand, Brazil, Mexico, and Chile. Research capabilities for the development, standardization and validation of diagnostic methods are also well advanced in these countries.

PCR-based diagnostics are increasingly used in developing countries for the early diagnosis of disease. However, their use is largely restricted to laboratories of research institutions and universities and to the central and regional diagnostic laboratories run by governments. Their use in field laboratories belonging to veterinary health authorities is basically non-existent. The participation of the private sector in animal disease diagnostics is restricted to the development and commercialization of kits, and that too in few developing countries such as India, China, Thailand, Brazil, Chile and South Africa.

Molecular epidemiology is one of the most powerful applications of gene-based technologies in animal health. PCR-based techniques are used in molecular epidemiology in some developing countries (for example, Brazil, China, India, Mexico, South Africa) to compare sequence data on PCR products to determine the genetic relationship of the disease-causing agents, thereby facilitating the determination of their source, monitoring their spread, and providing new information about their biology and pathogenicity. The information obtained from such investigations helps develop appropriate strategies for the diagnosis and control of diseases and to monitor the impact of disease control programmes. Molecular genetic analysis studies of rinderpest viruses have contributed substantially to the Global Rinderpest Eradication Programme (GREP). Similar studies on virus serotypes associated with FMD were useful for vaccination and control programmes in Asia (Madan, 2005).

Increased use of molecular-based diagnostics in developing countries has been possible due to the availability of reliable and affordable laboratory equipment and the increased support of international organizations such as FAO, IAEA and OIE, in providing training and post-training support services, regular proficiency testing, and giving increased emphasis on validation, standardization and quality control of diagnostic techniques.

Lately, the emphasis in training programmes and developmental projects, for example, those sponsored by FAO and IAEA, has been on quantitative or qPCR which requires less hands-on time than conventional PCR, is less labour intensive and more accurate, has a higher rate of throughput, obviates the need to handle post-PCR products, has a higher sensitivity and lower risk of contamination, allows quantitative estimation and uses multiplex diagnostics (multiple primers allowing amplification of multiple templates within a single reaction). The Joint FAO/IAEA Division has technical cooperation projects in 23 countries where qPCR is used as part of diagnostic services, and has held a number of training courses on biotechnology-based disease diagnostics tools for participants from many developing countries. The training has covered the diagnosis of brucellosis, fascioliasis and HPAI.

An EU-funded Consortium, FLUTRAIN, is also active in providing training to East European, Asian and African scientists in diagnostics and disease management tools. In January 2009, it provided training on diagnosis of HPAI to participants from Bangladesh, India, Morocco, Egypt and the Philippines. Currently, the National Veterinary Institute<sup>5</sup>, Uppsala, Sweden, an OIE Collaborating Centre, is planning hands-on training focusing on HPAI sequencing, bioinformatics and phylogeny to participants from Bulgaria, Hungary, Iran, Iraq, Macedonia, Namibia, Romania, Syria, Turkey, and Ukraine. WHO and FAO have also trained developing country scientists in molecular diagnostics in zoonotic and transboundary animal diseases.

Through a Joint FAO/IAEA Division Coordinated Research Project on the examination of methods to differentiate infected and vaccinated animals with FMD<sup>6</sup>, kits from many sources were examined in a network of laboratories. Thousands of sera were evaluated from many sources in order to validate the practical use of the kits. Such kits are now used routinely and are important in epidemiological decision making concerning whether countries or areas within countries are FMD virus free. This project also highlighted the cooperation between public institutions and commercial companies producing kits using non-structural proteins of FMD as target antigens.

The area of diagnostics is beset with problems of validation. Many competent diagnostic assays that are fit for their intended purpose exist, but to varying degrees may need to be validated and harmonized. International staff providing training on PCR-based

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<sup>5</sup> [www.sva.se/oie-cc](http://www.sva.se/oie-cc)

<sup>6</sup> [www-naweb.iaea.org/nafa/aph/crp/aph-fmdv.html](http://www-naweb.iaea.org/nafa/aph/crp/aph-fmdv.html)

methodologies are of the opinion that only 30 to 50 percent of laboratories in developing countries are using the techniques properly (J. Crowther and G.J. Viljoen; personal communication). One-time training is not sufficient and there is a need for after-training support services to most of laboratories. The challenges are most severe in those countries with a low knowledge base in biochemistry and in good laboratory practice and which lack a good laboratory infrastructure. Work to make PCR-based assays robust, to develop isothermal amplification methods (which do not require thermal cycling and result in a colour change that can be seen without the need for equipment), and on-site assays (e.g. pen-side tests, biosensors) (Belák, 2007), is ongoing. For example, efforts are underway to develop isothermal amplification-based assays for HPAI and PPR at the Joint FAO/IAEA Division<sup>7</sup>. Such developments would particularly enhance the possibility of accurate testing and reporting in developing countries where reporting systems and sending of samples are highly problematic. The Institute for Animal Health (IAH) in the United Kingdom has developed a pen-side diagnostic kit for rinderpest which uses eye-swabs and gives results in only five minutes. Field trials have been conducted in India and Africa.

Efforts to enhance human capacities in developing countries and countries in transition to use modern diagnostic methods will improve the capability of surveillance systems and disease control in these countries. International organizations are encouraged therefore to develop mechanisms and provide resources to train scientists to have the necessary skills to perform good research. Such capabilities will equip the scientific community to develop and adapt biotechnologies that meet local conditions and provide solutions to emerging and future problems.

### **Recombinant vaccines**

Immunization can be one of the most effective means of preventing and hence managing animal diseases. In general, vaccines offer considerable benefits at a comparatively low cost, which is a primary consideration for developing countries. Molecular techniques can be used to produce a variety of different constructs of pathogenic agents and offer several advantages over more conventional vaccines such as: the deletion of the gene(s) responsible for causing disease and thus greater safety; increased stability (which is an advantage for their effective use in developing countries); the possibility of developing vaccines against protozoan and helminth parasites; and differentiating between infected and vaccinated animals through detecting antibodies either against the peculiar proteins elicited by the vaccine or failing to detect antibodies against the deleted gene/protein (DIVA vaccines). However, few recombinant vaccines are being commercially produced (Table 3), and so far their use in developing countries is negligible.

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<sup>7</sup> [www-naweb.iaea.org/nafa/aph/stories/2009-avian-influenza.html](http://www-naweb.iaea.org/nafa/aph/stories/2009-avian-influenza.html)

TABLE 3

## SOME COMMERCIALIZED RECOMBINANT VACCINES

Target pathogen	Target animal	Brand name	Distributor	Characteristics
<b>Viral vaccines</b>				
Porcine circovirus type 2 (PCV2)	Pigs	Porcilis-PCV2	Intervet	Inactivated baculovirus expressed PCV2 ORF2 protein; adjuvanted
PCV2	Pigs	Suvaxyn PCV2	Fort Dodge	Inactivated PCV1-2 chimera; adjuvanted
Pseudorabies virus	Pigs	Suvaxyn Aujeszky	Fort Dodge	gE- and thymidine kinase-deleted marker vaccine
Classical swine fever virus	Pigs	Porcilis Pesti	Intervet	Baculovirus recombinant E2 protein without emulsion
Classical swine fever virus	Pigs	Bayovac CSF E2	Bayer Leverkusen	Baculovirus recombinant E2 protein without emulsion
Bovine herpesvirus type 1 (BHV-1)	Cattle	Bovilis IBR Marker	Intervet	Live or inactivated gE-deleted marker vaccine
Marek's disease virus (HTV) and infectious bursal disease virus	Poultry	Vaxxitek HVT+IBD	Merial	Live recombinant chimera virus expressing VP2 gene of IBD on HTV virus
Newcastle disease virus (NDV)	Poultry	Not applicable	Dow AgroSciences	HN recombinant produced in plant cell lines (registered but not on market)
Newcastle disease virus	Poultry	Vectormune FP-ND	Biomune	Fowlpox virus vectored
Avian influenza virus (H5N1) and NDV	Poultry	Not applicable	Intervet	Chimera virus on NDV backbone; field trials in 2007
Avian influenza virus	Poultry	Poulvac FluFend I AI H5N3 RG	Fort Dodge	Chimera H5N3 virus, inactivated in oil-based adjuvant
Avian influenza virus	Poultry	Trovac AI H5	Merial	Fowlpox virus-vectored H5
<b>Bacterial vaccines</b>				
<i>Actinobacillus pleuropneumoniae</i>	Pigs	PleuroStar APP	Novartis Animal Health	Recombinant ApxII, TbpB, CysL, OmlA(1), and OmlA(2) proteins
<i>Actinobacillus pleuropneumoniae</i>	Pigs	Porcilis APP	Intervet	Extracted Apxl, ApxII, ApxIII, and outer membrane proteins
<i>Salmonella</i>	Chickens, Hens	Megan Vac1 MeganEgg	Lohmann Animal Health International	Double gene-deleted <i>S. enterica</i> serovar <i>Typhimurium</i> strain
<i>Brucella abortus</i>	Cattle	RB-51	Colorado Serum Company CZ Veterinaria	Spontaneous rifampin-resistant rough mutant

Source: Meeusen et al. (2007)

Commercial tick vaccines: TickGUARD and Gavac vaccines against *Boophilus microplus* (Egerton, 2005)

The successful application of a recombinant DNA vaccine for the elimination of foot-rot disease in Nepal and Bhutan has been described, but was done on an experimental basis only (Egerton, 2005). In 1994, recombinant vaccines against *Boophilus microplus* were produced in Australia (TickGUARD vaccine) and Cuba (Gavac vaccine). Both vaccines

have been commercialized and tested in the field, e.g. in Argentina, Australia, Brazil, Cuba, Egypt and Mexico, and have been shown to be efficacious, although with some degree of variation (Willadsen, 2005). A killed subunit vaccine has been developed in Israel against coccidiosis in poultry. However, it is expensive to produce (Meeusen *et al.*, 2007). The University of California, Davis, United States, has developed a recombinant DNA vaccine against rinderpest and tested it in restricted conditions in Ethiopia. DNA sequencing and other molecular tools are in use at the University of Ibadan, Nigeria, in an effort to develop a vaccine for the prevention of infectious bursal disease, also known as Gumboro disease, which causes poultry deaths worldwide (Juma and Serageldin, 2007). Also in Africa, ILRI and the ARC-Onderstepoort Veterinary Institute, South Africa, are leading the way in the development of new vaccines.

According to an OIE survey (MacKenzie, 2005), 17 percent and 50 percent of African and Asian countries respectively produce or use animal vaccines that are biotechnologically derived (Table 4). Most of these countries are using vaccines produced in other countries rather than producing their own. In Africa, only one country reported using DIVA vaccine.

A recombinant capripox-rinderpest virus vaccine has been developed by the IAH and field trials are running in Kenya. Using the genome data of African swine fever virus, efforts to design, develop and test new vaccines are also underway at this institute<sup>8</sup>.

TABLE 4

APPLICATION OF BIOTECHNOLOGY-DERIVED ANIMAL VACCINES IN DIFFERENT PARTS OF THE WORLD

	Global	Africa	Asia	Middle East
Number of countries producing or using biotechnology-derived vaccines in animals	40 (44)*	4 (17)	7 (50)	1 (50)
Number of countries using viral-vectorized vaccines which include antigen(s) from unrelated organisms	26	2	4	0
Number of countries using bacterial-vectorized vaccines which include antigen(s) from unrelated organisms	16	1	5	0
Number of countries using vaccines which have deleted antigen(s) to differentiate infected from vaccinated animals (DIVA)	22	1	3	1
Number of countries using vaccines that include recombinant proteins	26	0	6	0
Number of countries using DNA vaccines	6	0	2	0
Number of countries using other products (undefined)	1	0	1	0

Source: adapted from MacKenzie (2005)

\* Values in parentheses are the percentage of countries that responded

<sup>8</sup> [www.iah.bbsrc.ac.uk/](http://www.iah.bbsrc.ac.uk/)

The DIVA technology has been applied successfully to HPAI and pseudorabies (Aujeszky's disease) eradication campaigns, and has been proposed for use in the eradication of CSF and FMD (Pasick, 2004). The DIVA-based vaccines for infectious bovine rhinotracheitis (IBR) and pseudorabies have been available commercially since the 1980s (Meeusen *et al.*, 2007). Work to develop marker vaccines against PPR and rinderpest is also in progress (Mahapatra *et al.*, 2006; Parida *et al.*, 2007; Diallo *et al.*, 2007). For CSF, the first DIVA-based vaccines were based on baculovirus-expressed E2 glycoprotein of CSF virus and have been marketed since 1993. However, these have the disadvantage of inducing a delayed immune response and are therefore not as effective as the conventional live attenuated vaccine. Various possibilities for the development of effective DIVA-based vaccines for CSF are discussed by Beer *et al.* (2007).

The first plant-based vaccine (recombinant viral hemagglutinin neuraminidase (HN) protein generated in plant cell lines via *Agrobacterium* transformation) for Newcastle disease virus in poultry could successfully protect chickens from viral challenge, but no product is yet on the market (Meeusen *et al.*, 2007). Recombinant vaccines have been developed that are highly effective in preventing infection with tapeworms: *Taenia ovis* in sheep, *Taenia saginata* in cattle, *Taenia solium* in pigs and *Echinococcus granulosus* in livestock (Lightowers, 2006; Eddi *et al.*, 2006). Since farmers must destroy meat from animals infested with tapeworm, the new vaccines could save farmers from huge economic losses.

In addition to validated, robust, specific and sensitive diagnostic tools and safe and effective vaccines, control and eradication of animal diseases requires a complete package of good veterinary infrastructure, reporting systems, laboratories with skilled staff, epidemiological units able to execute surveys, and a carefully designed plan with clear objectives. Regional and intergovernmental cooperation is also vital since many of animal diseases are transboundary.

### **Sterile insect technique**

The SIT depends on the integration of biological and engineering techniques to produce on an industrial scale and release, usually by air, adequate numbers of reproductively sterilized insects of the target pest in areas where it severely threatens the environment, agriculture or livestock production. Virgin female individuals in the target insect pest population that are mated and inseminated by released sterile male insects do not produce any offspring. Repeated inundative releases of mass-produced sterile insects can be integrated with suppression, eradication, containment or prevention strategies against key insect pests.

Trypanosomosis is a disease caused by blood parasites of the genus *Trypanosoma* and is transmitted in Africa by tsetse flies (*Glossina* spp). More than 30 tsetse fly species and subspecies infest an area of 8.7 million square km (approximately a third of Africa's total land area) and affect animals and humans in 35 sub-Saharan countries. The infection

threatens approximately 45–50 million head of cattle and WHO estimates that in the year 2000 some 50–60 million people in Africa were exposed to the bite of tsetse flies, which can result in sleeping sickness. There are situations where the SIT may be a necessary component of an AW-IPM approach for freeing areas under agricultural development from the trypanosomosis disease burden.

The SIT played a vital role in the eradication of the tsetse population of *Glossina austeni* from Unguja Island (Zanzibar) using an AW-IPM approach. The fly population was initially suppressed using insecticide-based control strategies such as stationary targets and pour-on insecticides for livestock. This was followed by the sequential aerial release of sterile males which drove the population to extinction, i.e. the last wild tsetse fly was trapped in 1996. Using data from 1999 as a baseline, an increase in average income per annum of farming households by 30 percent was recorded in 2002. Overall the quality of people's life improved substantially due to increased livestock and crop productivity, animal availability for transport and traction etc. In addition, the removal of the tsetse population from the Jozani forest reserve facilitated preserving this endangered habitat and removed a major threat to adjacent livestock and agricultural systems. Efficient wildlife management practices have also resulted in an increase in the numbers of some rare and protected wildlife species, such as the Zanzibar red colobus monkey, *Pilicolobus kirkii*.

The African Union's Pan-African Tsetse and Trypanosomiasis Eradication Campaign (AU-PATTEC) is coordinating various national programmes that aim to integrate the SIT for creating selected trypanosomosis- and tsetse-free zones in Ethiopia, Kenya, Senegal, Uganda, Tanzania and in a transboundary area in Mozambique, South Africa and Swaziland, (Feldmann *et al.*, 2005). The Joint FAO/IAEA Division supports this programme, providing in addition technical advice in Burkina Faso, Chad, Mali and Zimbabwe to assess whether the SIT can be used in these countries as part of AW-IPM campaigns<sup>9</sup>.

The SIT was also used to suppress, locally eradicate or prevent the (re-)invasion of two other livestock pest insects, namely the New World screwworm (NWS) fly, *Cochliomyia hominivorax*, and the Old World screwworm (OWS) fly, *Chrysomya bezziana*, which cause myiasis in warm-blooded vertebrates (humans, livestock and wildlife). The SIT has been used to eradicate NWS in North and Central America and Libya, as well as containing it along the Panama-Colombia border. Most of the South American continent, except Chile, is infested with NWS. Vargas-Terán, Hofmann and Tweddle (2005) have described the various steps needed for making this continent free of NWS. OWS is widely distributed on the Indian subcontinent, in sub-Saharan Africa, and in Southeast Asia, as far north as Taiwan Province of China and to

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<sup>9</sup> [www-naweb.iaea.org/nafa/ipc/field-projects-ipc.html](http://www-naweb.iaea.org/nafa/ipc/field-projects-ipc.html)

Papua New Guinea in the southeast. The SIT has been successfully tested against this species in Papua New Guinea and Malaysia. In late 2007, an outbreak of OWS flies was observed in Yemen that is threatening the livelihoods of people, either directly or through their livestock.

Biotechnological tools such as molecular markers are being used to study the degree of gene flow between various pest insect populations and provide indications on their relationship and potential isolation. This useful information about particular pest populations can lead to better planning of AW-IPM campaigns that may integrate a SIT component. At present, there are many uncertainties surrounding the production and use of transgenic insects due to instability of the insertion and expression of the transgene. In addition, it requires addressing public concerns and putting in place a regulatory mechanism to properly conduct a risk assessment (Robinson, 2005).

### **Bioinformatics**

Bioinformatics is the comprehensive application of statistics, biology and a core set of problem-solving methods for helping to understand the code and evolution of life as well as their implications. It deals with the use of information technology in biotechnology for data storage and warehousing and DNA sequence analysis. Bioinformatics has overarching implications in the areas of animal health, reproduction and nutrition.

The design of diagnostic tools, drugs and vaccines will rely increasingly on bioinformatic data through sequence analysis. Gene prediction and functional annotations play an essential role in this process. Developing countries can benefit hugely through such studies because much sequence information and many bioinformatic tools are publicly available and freely accessible. Furthermore, molecular immunoinformatic tools also have the potential to help scientists in developing countries to produce epitope-driven multigene synthetic vaccines. However, developing-country scientists are not skilled in this rapidly expanding area of biology, with the exception of very few countries. In India, web-accessible databanks such as the Animal Virus Information System, and tools to store and analyse information generated by molecular and genomic projects in livestock research are available. Strong linkages exist between information technology and the biotechnology sector. The Biotechnology Information System Network, a division of the Department of Biotechnology of India, has covered the entire country by connecting to more than 50 key research centres. India also has programmes to upgrade the skills of agricultural scientists from other Southeast Asian countries. The contribution of bioinformatics research is of growing importance in the study of life sciences in China and Brazil, while in Africa, ILRI is building capacity in this field through various training programmes. In addition to training, access to improved search engines, data mining programs and other tools to improve access through the Internet to a vast body of biomedical literature and sequence data is required in developing countries.

Although bioinformatics is discussed here in the context of biotechnologies for animal health, it is certain to play a major role in other sectors of livestock production. The cattle genome sequence has recently been completed (Elsik *et al.*, 2009) and the sequences of chicken, pig and sheep genomes are either already available or nearing completion. Bioinformatics has played an important role in these achievements. As stated elsewhere in this Chapter, genome sequence information can be exploited to enhance animal production and health in several ways. In the “post-genomic” era, it has innumerable applications in the areas of comparative, functional and structural genomics.

### 3.5 REASONS FOR SUCCESSES AND FAILURES IN LIVESTOCK BIOTECHNOLOGIES IN DEVELOPING COUNTRIES OVER THE LAST 20 YEARS

Some important factors affecting success or failure in the applications of biotechnologies in developing countries are listed in Tables 5, 6 and 7 for animal reproduction, genetics and breeding; animal nutrition and production; and animal health respectively. For the purposes here, it is not relevant to list specific technical factors for each of the animal biotechnologies that prevent their wider applicability because many papers are available in the literature providing this information. It is evident from the Tables that, with the exception of molecular diagnosis, the most advanced biotechnologies based on molecular biology are hardly used in developing countries, mainly because they are cost prohibitive, complex and require highly skilled personnel. The high cost of registering products such as new vaccines, probiotics and enzymes is another factor that limits their production in developing countries. The adoption of cloning and transgenesis is also affected by ethical, religious and animal welfare concerns. In addition, these two technologies and recombinant vaccine technology also need to be improved in terms of cost and efficacy in order to be of practical value.

The adoption of less advanced biotechnologies (e.g. progesterone measurement, oestrus synchronization, IVF and ET, cryopreservation, and semen and embryo sexing) has been low. This has largely been due to a combination of inadequate technical skills and infrastructure and inadequate profits for users. For example, liquid nitrogen is necessary for AI with deep-frozen semen and cryoconservation of genetic resources, but it is often costly when purchased commercially and requires a significant capital investment for on-site production. Factors such as slow speed of sorting, low sperm viability and low fertility rates (12 to 25 percent of that with conventional semen) together with the high cost of semen also limit the successful application of sperm sexing technology. Meanwhile, complicated IVF, ET and embryo freezing procedures and the low rates of success and high costs involved in producing embryos also constrain their wider adoption. The use of monensin and rBST is also affected by low public acceptance and by the lack of adequate or good quality feed

in developing countries. Although the production and use of prebiotics, probiotics and silage enzymes are relatively simple, technical constraints, especially insufficient knowledge about how to create the conditions that result in consistent positive responses are the limiting factors for their wide application, even in intensive production systems. Quality control systems and regulatory oversight of the products are non-existent. Silage making is common in developed countries, but has not been popular in developing countries due to a variety of factors including insufficient technical skills of farmers, extension activities and infrastructure and tools; also, in many countries the timing of silage making conflicts with other farm activities that are rated as more important. Silage additives will not be used in developing countries if silage preparation is not practised.

Although technologies such as single cell protein production and solid-state fermentation of lignocellulosic materials can be categorized as low-tech, they have practically not been used at all. The main reason for the failure in adoption of single cell technology is the high cost of production. The amount of biomass produced is small and the liquid volume in which it is produced large; the equipment required for removing water is expensive and the methods are time-consuming; and the energy needed for drying the isolated biomass also increases the cost. Furthermore, the biomass produced has a high nucleic acid content which limits its use in the diets of monogastric animals. The presence of high levels of nucleic acids in single cell protein also makes it a poor protein supplement for ruminants. The reasons for the failure of solid state fermentation of lignocellulosic materials such as straw are also the high cost involved in transport and processing of the straw before inoculation with white rot fungi, considerable loss in energy from lignocellulosic material during fermentation and difficulty in upscaling the process. The quality of the feed obtained after fermentation is not commensurate with the efforts and money spent. In short, the technology does not seem to be profitable.

Among the animal biotechnologies, modest success has been achieved only in the application of AI, molecular diagnostics and conventional vaccines, feed additives and the SIT.

## Artificial insemination

AI has played an important role in enhancing animal productivity, especially milk yields, in developing countries that have a well defined breeding strategy and a sound technical base to absorb and adapt the technology to meet their needs. Such countries also have: 1) an effective technology transfer mechanism for AI; 2) effectively integrated international assistance into their national germplasm improvement programmes; 3) built and maintained the infrastructure required; 4) complemented AI with improvements in animal nutrition and veterinary services; and 5) provided adequate economic incentives to their farmers by giving them access to markets and making sure that they get the right price for their products. Many other developing countries lack one or more of these requirements.

## Molecular diagnostics and conventional vaccines

In the area of disease diagnosis and control, most national governments have provided reasonably good policy and financial support, driven largely by the zoonotic nature of most of the diseases concerned. The availability of government support facilitated the development of the technical capabilities and physical infrastructure required. The international assistance obtained by developing countries in this field has been well integrated into their national programmes, leading to the realization of better adoption and higher impact. Furthermore, the impact of using these biotechnologies is easy to estimate economically based on projections of the number of animals prevented from dying or becoming diseased. This makes it easier for national and international agencies to quantify the impact of specific technologies and justify their programmes properly. This helps countries to raise more funds nationally and from donors, which in turn gives further impetus to the programmes.

The assistance of international organizations such as FAO, IAEA, OIE and WHO has contributed substantially to the success of biotechnologies such as AI, molecular diagnostics and conventional vaccines. They have facilitated training programmes to improve technical, analytical, and technology transfer skills, and provided financial assistance for building infrastructure, including state-of-the art laboratories. There is a strong positive correlation between the research capabilities of national biotechnological scientists and the scale of application of technologies in the field.

## Feed additives

The addition of nutrients and feed additives such as amino acids, enzymes and probiotics to the diets of monogastric animals is driven mainly by the increased benefit to cost ratio of these interventions, leading to greater profit of commercial livestock enterprises. The companies producing additives usually have skilled workers to advise farmers in preparing diets, as well as access to software for balancing protein requirements through the addition of amino acids. These factors have also been important for the success of these technologies. Another reason is that the production of additives is based on fermentation technology which has a long history of use in developing countries and is a low-cost intervention. The technologies have the added advantage of making the farms more environmentally sustainable by reducing pollution. In the near future, regulations on the release of nutrients such as nitrogen and phosphorus into water channels will increasingly be enforced in developing countries, which will further increase the adoption of the technology.

## Sterile insect technique

This technology is being applied along with a number of conventional approaches in a concerted manner. The reasons for its success in some places and failure in others have been critically examined (Vreysen, Gerardo-Abaya and Cayol, 2005; Alphey *et al.*, 2010). The SIT

projects supported by the Joint FAO/IAEA Division in Zanzibar and Libya were highly successful, as were many SIT projects in the area of crop pest control. On the other hand, the NWS programme in Jamaica showed that success cannot be taken for granted and that several prerequisites need to be in place. On the technical side, particularly important success ingredients are: the accurate and adequate collection of baseline data through the involvement of experts; the timely analysis of data; the development of sound operational plans and strategies; the delivery of extensive training to improve local expertise; the use of sterile males that are capable of competing with wild males for mating with wild females, and the availability of backup strains in case of loss of competitiveness in the field; the use of sound monitoring methods to evaluate the competitiveness of sterile insects; the availability of sound monitoring methodologies and their consistent use (use of different methods at the time of baseline data collection and during the SIT execution and monitoring phase could lead to wrong decisions being made). Equally important are meeting sound managerial and operational requirements which include: the presence of a flexible and independent management structure; the consistent availability of funds and trained staff; the presence of adequate expertise in the biology of the target insects and in the management of integrated projects; the strong commitment of all stakeholders, including though public awareness and education initiatives; an independent peer review system; consistency and continuity in the implementation of various components. Many of these are also critical to the success of applying other biotechnologies.

TABLE 5

**CURRENT STATUS OF ANIMAL BIOTECHNOLOGIES AND FACTORS INFLUENCING THEIR APPLICABILITY IN DEVELOPING COUNTRIES: ANIMAL REPRODUCTION, GENETICS AND BREEDING**

Biotechnology	Extent of use	Public and government acceptance	Current technical capability for using it	Current technical capability for adapting or developing it	Infrastructure and materials and tools available for its use	Relative cost	Skills required for application	Potential for generating impact (time frame < 10 years)
AI	Moderate	High	Moderate	Low	Moderate	Moderate	Moderate	High
Progesterone measurement	Low	High	Low	Low	Low	Moderate	Moderate	Moderate
Oestrus synchronization	Low	High	Low	Low	Low	Moderate	Moderate	Moderate
IVF and ET	Low	High	Low	Low	Low	High	High	Moderate
Molecular markers	Low	High	Low	Low	Low	Moderate	High	Low
Cryopreservation	Low	High	Moderate	Low	Low	Moderate	High	High
Semen and embryo sexing	Low	High	Low	Low	Low	High	Moderate	High
Cloning	Low	Low	Low	Low	Low	High	High	Low
Transgenesis	None	Low	Low	Low	Low	High	High	Low

TABLE 6

**CURRENT STATUS OF ANIMAL BIOTECHNOLOGIES AND FACTORS INFLUENCING THEIR APPLICABILITY IN DEVELOPING COUNTRIES: ANIMAL NUTRITION AND PRODUCTION**

Biotechnology	Extent of use	Public and government acceptance	Current technical capability for using it	Current technical capability for adapting or developing it	Infrastructure and materials and tools available for using it	Relative cost	Skills required for application	Potential for generating impact (time frame < 10 years)
Feed additives: amino acids, enzymes & probiotics	Moderate in intensively managed commercial monogastric farms; low in ruminant production systems	High	Moderate	Moderate	Moderate	Moderate	Moderate	High
Prebiotics	Low	High	Low	Low	Low	Moderate	Moderate	Moderate
Silage additives	Low	High	Low	Low	Low	Moderate	Moderate	Low
Monensin	Low	Moderate	Moderate	Moderate	Low	Moderate	Moderate	Moderate
Single cell protein	Low	High	Moderate	Moderate	Moderate	Moderate	Moderate	Low
Solid state fermentation of lignocellulosics	None	High	Moderate	Moderate	Moderate	Moderate	Moderate	Low
Recombinant somatotropin	Low	Moderate	Low	Low	Low	Moderate	Moderate	Moderate
Molecular gut microbiology	Low	High	Low	Low	Low	Moderate	High	Moderate

TABLE 7

**CURRENT STATUS OF ANIMAL BIOTECHNOLOGIES AND FACTORS INFLUENCING THEIR APPLICABILITY IN DEVELOPING COUNTRIES: ANIMAL HEALTH**

Biotechnology	Extent of use	Public and government acceptance	Current technical capability for using it	Current technical capability for adapting or developing it	Infrastructure and materials and tools available for using it	Relative cost	Skills required for application	Potential for generating impact (time frame < 10 years)
Molecular diagnostics	Moderate	High	Moderate	Low	Moderate	Moderate	High	High
Recombinant vaccines	None	Moderate	Moderate	Low	Low	High	High	High
Conventional vaccines	Moderate	High	Moderate	Low	Moderate	Moderate	Moderate	High
SIT	Moderate	High	Moderate	Low	Moderate	Moderate	High	High
Bioinformatics*	Low	High	Low	Low	Low	Moderate	High	High

\* This field is also relevant to animal reproduction, genetics, breeding, nutrition and production

## 3.6 CASE STUDIES OF THE USE OF BIOTECHNOLOGIES IN DEVELOPING COUNTRIES

### 3.6.1 Sustainable intensification of sheep rearing on the Deccan plateau in India<sup>10</sup>

Deccani sheep are reared traditionally in flocks of 20–200 ewes on the Deccan plateau in southwestern India by the Dhangar community in Maharashtra State as well as in the States of Karnataka and Andhra Pradesh. Sheep rearing is well integrated in the agricultural production system. Sheep graze on crop residues and grass along roadsides, farm bunds and canal verges. Sheep manure is sold to farmers at a remunerative price and is in great demand for cash crops such as sugarcane and orchards. Often sheep are penned overnight in farmers' fields. Sheep rearing communities earn a good livelihood but are socially disadvantaged with poor access to civic amenities and education. They rear sheep mainly to earn an income from selling lambs. The sale price of the coarse wool produced is usually not enough to cover the cost of shearing. Breeding rams are always with the ewe flock and mating is unplanned. Deccani ewes exhibit oestrus throughout the year with the possible exception of the winter months of January–February and the hot summer months of April to early May. Deccani ewes have only single offspring and lamb about every ten–twelve months. Flock owners sell the lambs in nearby markets on specified weekly market days when the lambs have reached about 3.5 months of age and 12–15 kg of weight. Lambs are sold on a per head basis and the price per kg live weight works out at 80–100 rupees (US\$1.6–2). The price of sheep meat has increased by 10–20 percent every year for the past several years.

Seventy percent of smallholder shepherds migrate during the dry season to areas with higher rainfall to find grazing and water for their sheep. The duration of migration varies from 3–8 months and the migration distance varies from 20–200 km. Grazing flocks are always shepherded and supervised closely, and the sheep are penned near the owner's house at night. It is common to cross-foster lambs to ewes or goat does that produce more milk than the dam. Lambs are valuable and even very young orphan lambs fetch a price. The profitability of sheep production is thus sensitive to the reproductive rate and even a modest increase would increase the owner's income substantially. Grazing land available for sheep is being lost steadily over the years due to erosion and other forms of degradation, increasing urbanization, industrialization and the expansion of irrigation and crop agriculture into marginal lands. Demand for sheep meat, however, is also increasing constantly. The sustainable intensification of sheep rearing to improve sheep productivity and efficiency could therefore be viable.

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<sup>10</sup> Contributed by Chanda Nimbkar, Animal Husbandry Division, Nimbkar Agricultural Research Institute, Phaltan, Maharashtra, India - April 2009; Nimbkar et al., 2009.

The *FecB* or Booroola mutation in sheep is an autosomal mutation in the bone morphogenetic protein receptor, type 1B, gene (BMPR1B) that has a large additive effect on the ovulation rate and is partially dominant for litter size. *FecB<sup>B</sup>* is the allele at this locus promoting higher fecundity while *FecB<sup>+</sup>* is the wild type allele. For ten years from 1998, the Nimbkar Agricultural Research Institute (NARI), an NGO, ran a series of projects funded by the Australian Centre for International Agricultural Research (ACIAR) to investigate ways of improving the performance of the local Deccani breed. The University of New England, Australia, the National Chemical Laboratory (NCL), Pune, India, and the University of Melbourne, Australia were major collaborators in the projects. One of the initiatives was the introduction of the *FecB<sup>B</sup>* mutation from the small, prolific Garole sheep (adult ewe weight 12–15 kg) of Sunderban in West Bengal State into the Lonand strain of the Deccani breed (adult ewe weight 28 kg) followed by backcrossing based on the *FecB* genotype in order to improve prolificacy while retaining the larger size, local adaptation and meat producing ability of the Deccani breed. A composite strain of Deccani, Israeli Dairy Awassi and Bannur was also produced with the *FecB<sup>B</sup>* mutation introduced from the Garole to benefit from the larger size and superior milking ability of the Awassi and the meaty conformation of the Bannur. Crossbred *FecB<sup>B</sup>* carrier ewes and rams were disseminated into local shepherds' flocks. However, after the first introduction of ewes, further dissemination was only through rams due to adaptation problems associated with the ewes. Additionally, 40 *FecB<sup>B</sup>* carrier rams were purchased for breeding by individual sheep owners, NGOs and State governments from Maharashtra and five other states.

One copy of *FecB<sup>B</sup>* led to an increase in the ovulation rate from 1.0 egg to 2.0 eggs and an increase in live litter size at birth from 1.0 to 1.6 in the NARI flock and from 1.0 to 1.4 in smallholder flocks. Litter size of homozygous ewes was similar. Thus, only about 40 percent of the *FecB* carrier ewes in smallholder flocks had twins and less than 5 percent of the litters of carrier ewes were triplets. The increased litter size was found to be moderate and manageable under the existing production system of smallholders. The small changes in management with increased twinning in smallholder flocks included keeping young lambs behind in the pens when ewes were grazing and providing lambs with a small amount of supplementary feed. Compared with 0.9 lambs of three months of age weaned by non-carrier ewes, *FecB<sup>B</sup>* ewes weaned 1.3 and 1.2 lambs in the NARI and smallholder flocks respectively. This was a 33 percent increase in productivity and income for a negligible amount of extra expenditure on feed and some extra care. A higher gain in productivity and income is expected from the progeny of the more recent batches of *FecB<sup>B</sup>* carrier rams sent to smallholder flocks as they are the products of more generations of backcrossing, leading to a smaller Garole proportion, a larger size and more of the phenotypic features

desired by smallholders. Smallholders were given free veterinary care and sheep insurance for the first four years. Training in ewe and lamb management and health care has been an integral part of the projects since the beginning.

The phenotype of *FecB<sup>B</sup>* carriers (increased number of ovulations and lambs) cannot be measured in males nor in females before the age of puberty, and is not completely associated with genotype in females (a female with two lambs is more likely to carry the *FecB<sup>B</sup>* mutation but often will not be a carrier and carrier ewes do not have twins at every lambing). The DNA test for *FecB<sup>B</sup>* detection was therefore established under the project at NCL.

There are now 13 homozygous and 240 heterozygous adult ewes in 16 smallholder flocks which were born in these flocks. Some shepherds have retained heterozygous rams born in their flocks for further breeding. NARI will continue disseminating *FecB<sup>B</sup>* carrier rams in these and other flocks under a newly funded project from the Indian Government's Ministry of Science and Technology. Under the new project, the DNA test for *FecB<sup>B</sup>* detection will be set up at NARI and cost-effective management techniques for ewes and lambs will be investigated under smallholder flock conditions.

Twining was thus introduced successfully into non-prolific Deccani sheep from the Garole breed by introgressing the *FecB<sup>B</sup>* mutation with the help of the direct DNA test for detecting the animal's genotype at the FecB locus. NARI is the agency maintaining the nucleus flock and carrying out the genotyping and extension in smallholder shepherds' flocks. Genetic improvement is permanent and is therefore the best technology to improve the productivity of smallholder flocks in remote areas. For additional discussion of this case study, see Nimbkar (2009b).

### 3.6.2 The Global Rinderpest Eradication Campaign<sup>11</sup>

Rinderpest (cattle plague) is an infectious viral disease of cattle, buffalo, yak and numerous wildlife species that has caused devastating effects throughout history. In the 1890s, rinderpest destroyed nearly 90 percent of all cattle in sub-Saharan Africa and millions of wild animals. Major rinderpest outbreaks last approximately five years and have an average of 30 percent mortalities in a population. This poses a massive risk to millions of small-scale farmers and pastoralists. Major outbreaks of rinderpest could destroy more than 70 million (or 14 million per year) of the 220 million cattle in Africa. With an estimated value per head of US\$120, the cost of such an outbreak would be more than US\$1 billion per year and a total of US\$5 billion, based on an average outbreak lasting five years.

Today, the world is nearly free from rinderpest. Eliminating rinderpest could be viewed as producing a net annual economic benefit to the African region of at least US\$1 billion.

<sup>11</sup> Sources: [www-naweb.iaea.org/nafa/aph/stories/2005-iaea-rinderpest.html](http://www-naweb.iaea.org/nafa/aph/stories/2005-iaea-rinderpest.html) and John Crowther, Joint FAO/IAEA Division; April 2009

The only evidence of the disease surviving refers to a small focus in the Somali pastoral ecosystem that encompasses north eastern Kenya, southern Somalia, and some areas of Ethiopia. The goal of achieving complete freedom from rinderpest from the world is within reach. Its elimination would mark only the second time in history a disease has been eradicated worldwide, the first being smallpox.

The progress towards eradication through large-scale vaccination and surveillance campaigns has been a remarkable triumph for veterinary science. It serves as a powerful example of what can be achieved when the international community and individual national veterinary services and farming communities cooperate to develop and implement results-based policies and strategies. The key local coordinating institutions in the battle against rinderpest have been the Pan African Rinderpest Campaign (PARC) and later the programme for Pan-African Control of Epizootics (PACE), overseen by the African Union. FAO has provided support by serving as the Secretariat of the GREP, while the Joint FAO/IAEA Division provided technical expertise to projects funded by the Technical Cooperation Department of the IAEA.

The initial live vaccine developed by Walter Plowright and colleagues in Kenya with support from the United Kingdom, was based on a virus that was attenuated by successive passages in tissue culture, and he was awarded the World Food Prize in 1999 for this work. Although this freeze-dried live vaccine is highly effective and safe, the preparation loses some of its effectiveness when exposed to heat. Further research was directed at developing a more thermostable vaccine for use in remote areas and success was achieved through research in Ethiopia by Jeffery Mariner supported by the United States Agency for International Development (USAID). One of the striking features of the planning for the latest campaign was the total lack of foresight into the need and use of diagnostics. Although the vaccine side was well catered for (supply), the estimation as to whether vaccines worked (whether antibodies were produced so estimating whether cattle had actually been vaccinated) and whether cattle were immune (the level and relevance of antibodies produced) were not initially addressed in scientific or financial terms.

The task of rescuing this situation fell on the IAEA and certain national institutions such as the IAH and the Institut d'Elevage et de Médecine Vétérinaire Tropicale in France. Basically, serological assays involving ELISA were developed to provide kits for the estimation of anti-rinderpest antibodies in cattle, and to determine also whether animals had antibodies against PPR, the equivalent of rinderpest in sheep and goats. The latter was necessary to sort out the complicated epidemiology of PPR and rinderpest in all species. Then the science of the epidemiology was necessary to allow an accurate assessment of the campaign's success. Later developments involved producing molecular-based methods for the identification and differentiation of rinderpest and PPR. This work allowed the unequivocal determination of

PPR, or ruling out rinderpest, in cases where clinical signs were compatible with presence of either disease. Along with ELISA for antibody detection there were developments of pen-side tests for detecting rinderpest and PPR antigens from eye swabs.

The combined technologies of serology and PCR produced a battery of tests able to specifically assess vaccine efficacy and to differentiate true rinderpest from PPR. Sampling frames were also important as they provided the statistical framework on which success was measured, and these were developed by FAO and IAEA with support from the Swedish International Development Cooperation Agency (SIDA). Along with the supply of tests came quality assurance methods (charting) to allow continuous assessment and external validation of methods (both vital in the long term for laboratory assurance). Such an armoury has permitted many countries to obtain official recognition of freedom from rinderpest according to the provisions of the OIE's international standards. Rinderpest disease is now no longer observed in the world. This status is assured through serosurveillance and other monitoring and by well trained personnel using methods which are of the correct diagnostic sensitivity and specificity to allow the results to be assessed statistically.

Although the cost of vaccination, blood sampling and testing have been high for both developing and developed nations, their effectiveness is demonstrated by the fact that there is only one small focus of virus with the potential to generate disease outbreaks left in the world. By contrast, in 1987, the disease was present in 14 African countries as well as in western Asia and the Near East. The economic impact of these efforts is already clear. Although the costs and benefits have varied considerably from country to country, the figures for Africa mentioned above illustrate the cost-effectiveness of the control measures implemented.

### 3.6.3 **Oestrus synchronization and artificial insemination in buffaloes in Punjab, India<sup>12</sup>**

The buffalo is an important component of Indian livestock and contributes around 50 million tons of milk and 1.5 million tons of meat annually, in addition to high valued hides, bones and draught power for agricultural operations. Compared with cattle, however, the buffalo is a slow breeder owing to its delayed puberty (around 36 months), and has a high incidence of suboestrus (20-80 percent) and prolonged postpartum anoestrus (>60 days), resulting in prolonged calving intervals. Interventions to improve fertility and production that are commonly used in dairy cattle have remained ineffective due either to species differences (suboptimal response to various endocrine treatments in buffalo) or to the impracticality of the smallholder farming systems prevalent in India (1-5 animals owned by each farmer).

Total AIs performed in Punjab rose from 1.9 million to 2.8 million between 1998 and 2005 (DADF, 2006). Although buffaloes in Punjab outnumber cattle (six million compared with two million), only 5 percent of buffaloes are bred using AI compared with 45 percent of

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<sup>12</sup> Contributed by P.S. Brar and A.S. Nanda, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab, India; April 2009

cows. Poor expression of oestrus, especially during summer (ambient temperature 35–45 °C and a severe lack of green fodder), and poor conception rates following AI have been the major deterrents. The synchronization of oestrus with progesterone and/or prostaglandins followed by fixed-timed AI (FTAI), commonly practised for dairy cows, failed to give the expected results in dairy buffalo, probably due to induced ovulations being inconsistent with too long a time spread. Therefore, there was a need to shorten the “ovulation window” following synchronization to improve fertility in dairy buffalo.

In Punjab, most buffaloes are bred through natural service by using any available bull, very few of which are progeny-tested or evaluated in any way. The genetic potential of buffaloes has therefore seen no discernible increase over the years. An effective protocol that would induce precision in ovulations, increase conception rates and improve progeny through the use of higher potential germplasm could substantially enhance the reproductive efficiency of buffalo. With these objectives, an “ovusynch” protocol was developed for buffalo to improve their fertility following AI. Ovusynch refers to the use of a set of hormones to synchronize oestrus and ovulation followed by FTAI. Extensive studies involving ultrasonographic, endocrinological and clinical observations on cycling buffalo were initiated in 2003. An effective ovusynch protocol was established in 2005 on the basis of the most probable time of ovulation and the best fixed time for AI that would yield acceptable conception rates.

The protocol consists of intramuscular injection of 20 µg of buserelin on the first day of the treatment, 500 µg cloprostenol on day seven and 10 µg buserelin on day nine (~60 hours after an injection of cloprostenol). Postpartum (>60 days) suboestrous buffaloes which remain unbred due to various reasons are selected. They are inseminated at 16 and 40 hours after the second buserelin injection irrespective of the expression of oestrus. Semen from proven and pedigree bulls of known fertility and genetic superiority is used. Following this treatment, approximately 67 percent of buffalo conceive in winter and 30 percent in summer. If they are supplemented with monensin (200 mg/buffalo/day for 30 days) before the start of the ovusynch application, the conception rate in summer is increased to 60 percent.

Multiple outreach activities are being undertaken to extend the technology for the genetic improvement of farmer-owned buffalo:

- *Pilot Projects:* Twelve pilot farms, involving 700 buffalo have been established in rural Punjab. Up to 70 percent of the enrolled buffalo conceived with semen from progeny tested bulls.
- *Training of Trainers:* Under the auspices of the Centre for Advanced Studies in Veterinary Gynaecology and Reproduction of the Indian Council of Agricultural Research, New Delhi, around 25 scientists from nine Indian states, 75 veterinary staff from the Punjab State Animal Husbandry Department and two international fellows from Mongolia and Myanmar have been trained on the application of ovusynch.

- *Linkages with NGO:* The Dr A.S. Cheema Foundation Trust, Chandigarh, India, is actively involved in the promotion of livestock production in rural areas in north India. The Trust is also bringing the technology to a large number of farmers in various districts of Punjab and the adjoining States of Haryana and Himachal Pradesh through its well established outreach activities.
- *Extension services of the Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, India:* The extension services of the University are disseminating this technology through the “Lab-to-Land Programme”, which consists of field services, field days and other animal health programmes. A conservative estimate would suggest that around 1 000 farmers and 5 000 buffalo have benefited from this programme to date. Of these, 60–75 percent of the buffaloes would have remained unbred for a variable period of 6–12 months in the absence of these efforts. A close follow-up of about 100 heifers produced through this programme at some of the pilot farms revealed that the female buffaloes produced under this study attain puberty at <28 months, compared with an average of >36 months for the state. The intervention led to an increase in milk production and provided additional calves of improved genetic potential to farmers by decreasing the calving interval and the age of first calving in heifers.

Ongoing wider adoption of this technology would contribute substantially to improving dairy buffalo production and benefit the economic situation of the farmers in India and in other buffalo-rearing countries.

#### 3.6.4 Community-based artificial insemination, veterinary and milk marketing services in Bangladesh<sup>13</sup>

Bangladesh has the largest population density in the world and most of its population is rural, with a per capita income among the lowest in the world. This population is continually growing, increasing the demand for food including animal products. Agriculture has evolved in an attempt to meet this demand. The purpose of rearing cattle has been shifting from their utilization as traction to milk and meat producing animals. AI was introduced in 1969 to help contribute to increase productivity but growth rates in production have lagged behind increases in consumption.

At the beginning of the 1990s, the Government of Bangladesh began a programme for small-scale dairy farming which led to a growth rate of 5.6 percent in the industry by 1995. The programme included the use of AI and crossbreeding for introducing germplasm from higher-producing exotic breeds. Farmers initially procured a large number of crossbred

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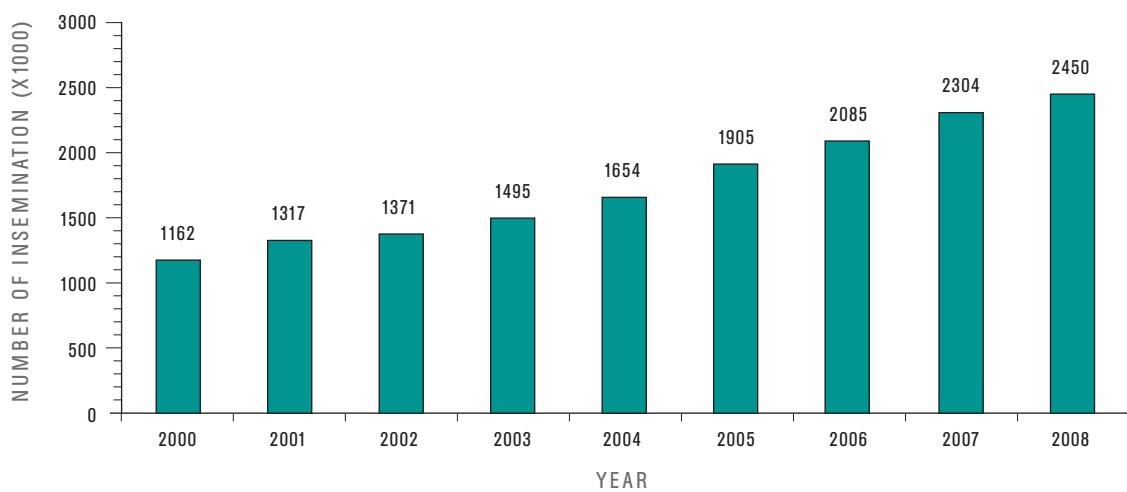
<sup>13</sup> Contributed by Mohammed Shamsuddin, Bangladesh Agricultural University, Mymensingh, Bangladesh; July 2009

cows through the popular AI services. However, the initial programmes were not all sustainable and the growth rate dropped sharply to 2.6 percent in 1997. Poor or non-existent opportunities for milk marketing and a lack of veterinary services to help manage the potential for increased productivity were the major causes for the lack of sustainability. The programme fared better in peri-urban areas with easier access to inputs and services and in areas where cooperatives such as the Bangladesh Milk Producers' Cooperative Union Limited operated milk collection and service delivery activities. It was concluded that AI and crossbreeding could contribute to improving dairy productivity and the incomes and livelihoods of farmers, but had to be complemented with other services to maintain the health and fertility of high-producing cows and to provide a good market for the increased volume of product.

Complementing AI with other services has helped increase its adoption, contributing to a doubling of the number of inseminations over the last nine years (Figure 1). About three million crossbred cattle are now in Bangladesh, representing 13 percent of the population. Two major players operate AI field services with semen produced from their own bull stations: the Department of Livestock Services, a public organization, and the AI Programme of the NGO Building Resources Across Communities (BRAC). AI in buffalo has also been introduced recently through an IAEA Technical Cooperation project.

FIGURE 1

**NUMBER OF INSEMINATIONS IN BANGLADESH FROM 2000 TO 2008**



Crossbred animals generally perform well, assuming that veterinary services are included in the AI programme and milk marketing opportunities are made available. Veterinary services are required because the crossbred cattle tend to suffer more from health and reproductive problems than local animals. Crossbred cows also require more inputs in feed and health care, so an available market is necessary to allow the farmer to obtain the revenue to cover these increased costs.

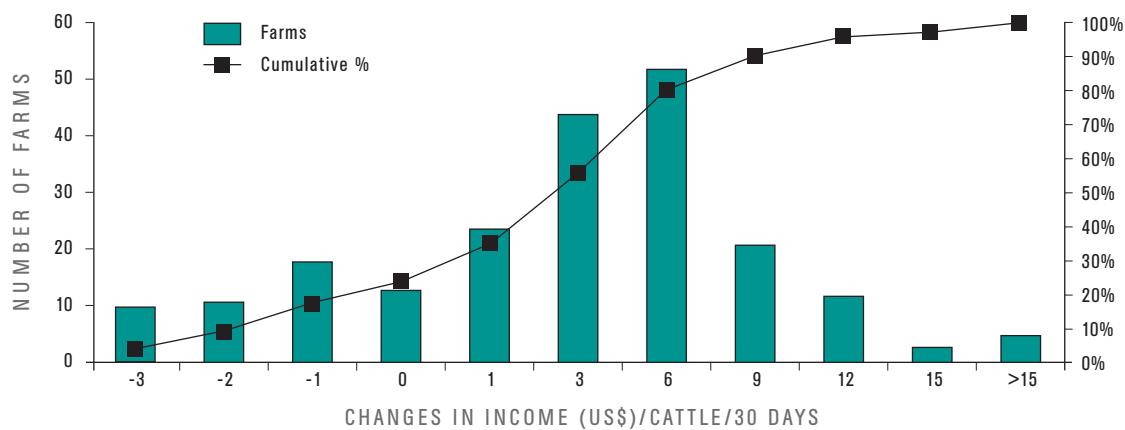
The impacts of such comprehensive AI programmes were evaluated in two districts of Bangladesh, Satkhira and Chittagong. In Satkhira, farmers were offered the opportunity to crossbreed their local cows with semen from a local AI programme. At the same time, a community-based dairy veterinary service (CDVS) was offered. Finally, a milk processor, BRAC Dairy and Food Projects, installed milk chilling tanks in the community. The CDVS is delivered through farmers' groups and associations which have laid the foundation towards operating the programme as self-financed. Three such associations collect about 7 000 litres milk per day and transport it to five BRAC milk chilling centres. BRAC also pays 1.65 Bangladeshi taka (approximately US\$0.024) for each litre of milk to the CDVS in addition to the milk price paid to producers, yielding a yearly income of approximately US\$62 000 – enough to pay the salary of three veterinarians, one field assistant, rents for three veterinary offices and the cost of vaccines and anthelmintics for all animals of the farm community. In addition, 69 men are employed to collect the milk and transport it to the BRAC chilling centres. Each man works two to three hours a day and earns at least US\$20 a month. The programme generates a large amount of off-farm employment, which is very important in a country like Bangladesh where unemployment is high.

A typical pattern observed is for farmers to use crossbreeding and improved veterinary services initially to increase the milk yield per cow. Over time, this allows farmers to accumulate funds and increase the number of cows. This has led to increases on single farms ranging from 35 to 90 times in total milk production and allowed farmers to become solvent members in the community. According to a recent economic analysis, the CDVS has tended to increase net income as well (Figure 2). More than 75 percent of farm families benefited from an increase in net income by using the services of the CDVS, with increases ranging from US\$1.0–US\$19.2 per cow per month.

A similar programme was established in Chittagong in 2002. At the beginning, there were 70 farmers producing about 1 500 litres of milk per day. Currently, the programme involves 210 farm families that collectively produce about 6 000 litres per day. In addition, the CDVS developed a farmers association that negotiates the milk price with the dairy sweetmeat industries. Prior to this, farmers used to be exploited by middlemen and sweetmeat producers. Now that productivity veterinary services and AI are available and the associations guarantee a reasonable price for milk, both the number of dairy farmers and milk production per farm have increased.

FIGURE 2

### EFFECTS OF PRODUCTIVITY VETERINARY SERVICES ON FARMERS' NET INCOME IN SATKHIRA



minimum and maximum differences were US\$-8.0 and 19.2 (number of farms = 213)

For sustainable continuation of the programme, the Bangladesh Agricultural University has created the Community-based Dairy Veterinary Foundation. The Foundation, in collaboration with farmers' associations and dairy processors, will run the programme without financial support from the university. The keys to the success of the programme are the inclusion of a dairy processor to ensure the marketing of the milk produced by the farm community and the availability of AI services.

#### 3.6.5 Assisted reproductive biotechnologies for cattle in Brazil<sup>14</sup>

During the last 40 years, the application of reproductive biotechnologies in the livestock sector of Brazil has experienced several phases of development in which methods were adapted, improved, substituted or added. Specifically in regard to the cattle industry, the major livestock sector in Brazil with around 200 million head, the 1970s were marked by the consolidation of AI use on a commercial scale. The use of frozen semen through AI programmes allowed the massive introduction of selected bulls of high genetic potential into different agro-ecological zones in the country, leading to an overall increase of production.

However, this success was limited in some cases by the fact that different *Bos taurus* breeds were introduced into tropical or semi-arid regions without proper monitoring of their capability to tolerate heat and resist parasitic infestation, resulting in unsustainable production systems. At that time, the recognition of zebu (*Bos indicus*) as ideal breeds for Brazilian tropical environments (they were originally imported from India in the 1920s and

<sup>14</sup> Contributed by José Fernando Garcia, Animal Production and Health Department, São Paulo State University, UNESP, Araçatuba, Brazil

1930s and then again in the 1960s), led to the establishment of several AI centres dedicated to the collection and distribution of semen from better adapted breeds, especially Nellore and Guzerat for beef production and Gir for dairying. In parallel, breeding programmes through breeders' associations and agribusiness groups were established, which played a pivotal role in the dissemination and monitoring of germplasm.

In the 1980s, when AI was increasingly being used, a second phase started, namely the use of multiple ovulation and embryo transfer (MOET) methods. Since then, Brazil has become one of the major users of this biotechnology (Garcia, 2001).

Recent data from the International Embryo Transfer Society indicate Brazil's leading position in South America in the use of embryo technology (Thibier, 2008; Table 2 earlier). In the 1990s, *in vitro* embryo production (IVEP) was taken from the laboratory to the field and emerged as one of the advanced technologies to solve specific bottlenecks in the use of bovine embryos for breeding purposes, namely, the lower response of zebu cows to ovarian stimulation with hormones and the rapid increase in market demand for high quality animals. This method can exploit the best of both male and female genetic potential and produce large numbers of descendants from the same specific artificial mating. One superior cow can have both ovaries submitted to monthly transvaginal ultrasound follicle aspiration, generating a large number of oocytes and producing on average more than 50 descendants per year. Of the approximately 820 000 bovine embryos transferred in the world in 2007, almost 30 percent were from Brazil, with about 46 000 being produced through MOET and 200 000 through IVEP. More than 90 percent of these were from zebu beef breeds. The use of IVEP was non-existent in Brazil until only ten years ago, but the current production represents about 95 percent of the total transferrable embryos produced *in vitro* in South America and about 50 percent in the world (Thibier, 2008).

Another recent development has been the increased application of FTAI, which has allowed large-scale application of AI in the beef sector. During the last decade, Brazilian scientists and pharmaceutical industries working in close partnership, developed a method consisting of the treatment of beef heifers or cows with specific hormone combinations to synchronize ovulation, allowing their insemination at one time. This revolutionized the use of AI even in areas where the infrastructure is not well developed and there is a dearth of highly skilled technicians because AI can be performed on a large number of animals in a single day by a qualified technician without oestrus detection (Baruselli *et al.*, 2004). The cost of the entire procedure is low (between US\$7–10 per treated cow). According to data from the Brazilian Association of Artificial Insemination, around eight million doses of semen were sold in 2007, with consistent growth during the last five years as FTAI has spread year after year and largely replaced conventional AI.

The combined use of AI, MOET, IVEP and FTAI in Brazil coupled with infrastructural development and overall nutrition, health and sanitary improvement has allowed fast distribution of animals having superior genetic attributes and opened new avenues for putting

in place well structured production chains which now benefit the country's economy. This integrated approach has created the basis for cattle population growth and contributed to elevating the productivity of both the beef and dairy sectors, stabilizing meat and milk prices, increasing food consumption per capita and positioning Brazil as a top meat exporter and a self-sufficient producer of milk (Table 8).

Unfortunately, the neglect of grassland management and the increase of deforestation have constantly been associated with the development of the cattle sector in Brazil, particularly with regard to beef production. The mitigation of the negative environmental effects of cattle production is becoming mandatory for the continuation of this sector. This requirement is forcing major changes in the organization of the cattle production chain to comply with the strict new environmental protection legislation. Cattle in Brazil occupy about 200 million ha of agricultural land and the major challenge now for the livestock sector in the country is to increase productivity while simultaneously releasing 100 million ha for other forms of agriculture production in order to prevent deforestation. According to recent data, in 2009 deforestation in Brazil reached its lowest level for the last 20 years, indicating the effectiveness of the measures adopted.

In conclusion: Brazil has experienced dramatic developments in the cattle industry in which the excellence of zebu breeds for tropical production systems has been exploited using assisted reproductive technologies. These biotechnologies have accelerated the spread of improved germplasm and played an important role in the economic development of the country. Brazil's research and technology in this area now equals that of developed countries. As a result of combining well-adapted germplasm to the environment, the prevailing technical competence and recent advances in genomic research, it is expected that zebu breeds and hybrids (especially the Nellore, Gir, Guzerat, Brahman and Girolando breeds) will emerge as promising options for cattle development in tropical countries, making Brazil an important player on the international cattle genetics market.

TABLE 8

CATTLE MEAT AND MILK PRODUCTION RECORDS AND FACTS FROM BRAZIL (1970-2007)

	Meat production (ton) <sup>*/**</sup>	Consumption (kg/person/yr)*	Meat Price (US\$/ton)*	Meat Exports (US\$Mio)**	Milk Production (ton)*	Milk Price (US\$/ton)*
Year	1970	1970	1994	1994	1970	1994
	1 845 182	17	1 800	573	7 353 143	254.97
Year	2007	2003	2006	2008	2004	2006
	9 296 700	33	1 550	5 500	24 202 409	221.81
Change (percent)	+500	+94	-20	+960	+350	-10

Sources: \*FAOSTAT (<http://faostat.fao.org/>) and the \*\*Brazilian Institute of Geography and Statistics (IBGE, [www.ibge.gov.br/home/](http://www.ibge.gov.br/home/))

## B. LOOKING FORWARD: PREPARING FOR THE FUTURE

### 3.7 KEY UNSOLVED PROBLEMS IN THE LIVESTOCK SECTOR WHERE BIOTECHNOLOGIES COULD BE FUNDAMENTAL TO THEIR SOLUTION

Continued population growth and urbanization, global warming, globalization of trade and the ongoing intensification of livestock production systems and value chains, in addition to providing opportunities for development, have given rise to a number of new challenges in animal production and these trends and new challenges will continue in the future. The challenges include the occurrence of new diseases, such as HPAI caused by virus of the H5N1 sub-type and, more recently, influenza A/H1N1, the re-occurrence of many old transboundary animal diseases, the release of pollutants such as methane, nitrogen and phosphorus into the environment, water scarcity, land degradation, the erosion of animal biodiversity and the scarcity of feed (due to the need to feed a growing population or because of diversion to other uses, such as biofuels). Animal biotechnologies provide opportunities for addressing new challenges and solving upcoming problems.

#### Control of new and (re-)emerging diseases

The emergence of vector-borne diseases such as African swine fever, bluetongue, Rift Valley fever and African horse sickness in new areas which is linked to global warming, is an increasing threat worldwide. The breaking down of borders between many countries, increasing international trade in live animals, animal products and feeds, and increasing wildlife-human interactions promoted by global climate changes are also contributing to new high-risk situations. For African swine fever there is no effective vaccine available and new variants of the virus have emerged in Africa, while in Sardinia it is present in endemic form. The infectious agents could appear in unexpected and unknown areas which may lead to improper or delayed diagnosis and result in the uncontrolled spread of the agent to large areas. These situations require sustained surveillance over the spread of diseases throughout the world. For example, the emergence of the West Nile virus in Europe and the United States requires continuous surveillance and a control programme for the presence of the virus in birds, horses and humans (Hayes and Gubler, 2006). New diseases such as Hendra virus, Nipah virus and severe acute respiratory syndrome coronavirus (SARS) demand continuous surveillance of wildlife for potential disease risks. Given that many of the emerging diseases worldwide are zoonotic, the risk to humans and animals and animal productivity could be better managed through the application of recent biotechnology-based diagnostics such as qPCR methods, microarrays, nucleic acid fingerprinting, DNA sequencing, biosensors, isothermal amplification methods and pen-side tests. These are

powerful techniques that enable the rapid, accurate and sensitive detection and identification of variants of the pathogens. The availability of effective DIVA-based vaccines is likely to increase in the future. This would also facilitate the control and eradication of transboundary animal diseases, including zoonotics.

Lately, PPR became a much more prominent disease because, apart from causing disease in small ruminants, it also impacted on the diagnostic and vaccination work for preventing rinderpest in large ruminants. The PPR virus can produce subclinical infection in large ruminants and the antibodies thus produced cross-react with the rinderpest virus and cause confusion in the diagnosis, with important implications for the campaign to eliminate rinderpest. Additionally, in areas declared free of rinderpest, the rinderpest virus strain cannot be used to vaccinate against rinderpest or PPR. The problem can be solved using molecular techniques such as DNA sequencing and through the development of a PPR marker vaccine.

Poultry and wildfowl have been considered as the major carriers of the HPAI H5N1 virus, and thus of the disease. However, recent data have demonstrated that both wild and domestic cats can carry the HPAI virus and may present a source of disease for humans (Kuiken *et al.*, 2004). Pigs are susceptible to both human and avian influenza viruses and it is speculated that co-infection of pigs with HPAI virus and human influenza virus may create viral reassortant strains with the ability for human-to-human transmission (Cyranoski, 2005). PCR-based and DNA sequencing methodologies have been central for genetically characterizing strains of H5N1 viruses. Similarly, for the ongoing outbreak of influenza A/H1N1, these techniques have been invaluable for characterizing the influenza virus and establishing that the virus circulating in the United States and Canada is the same as that in Mexico. Furthermore, using molecular techniques this virus has now been completely sequenced, which will help to pinpoint its origin, spread and change over time, and explain the differential and severity of disease between Mexico and the rest of North America.

The danger of bioterrorism is also looming. The emerging challenges cannot be met effectively without the use of molecular tools. Molecular diagnostics and molecular epidemiology have played and will keep on playing an essential role in detecting pathogens and preventing natural and bioterrorism-induced pandemics. The role of DNA marker vaccines will also be vital in providing a secure and productive environment for animal agriculture to flourish. The ongoing genomic studies for gaining insight into host-pathogen interactions are likely to produce novel and more effective approaches for diagnosis and control of diseases.

### **Efficient utilization of forages, global warming and land degradation**

Climate change is currently an issue of critical importance on the global stage. Livestock production has been implicated as substantially contributing to climate change as well as other types of environmental degradation (FAO, 2006a). Biotechnologies could play a role

in alleviating the impact of livestock on the environment. In the area of animal nutrition, the ongoing efforts to sequence the genomes of predominant rumen bacteria and assign functions to genes provide the opportunity to extend our understanding of gastrointestinal microbiomes beyond the degradative and metabolic characteristics relevant to both host animal health and nutrition. This facilitates acquiring the knowledge of a bacterium's competitiveness and colonization potential in the rumen and of the nutrient requirements of microbes, underpinning the roles of microbes in the process of feed digestion, and understanding better the mechanism of fibre degradation in the rumen. This knowledge may provide new opportunities for using roughages and crop residues more effectively and for developing strategies to achieve sustainable decreases in methane production through new means, one of which could be through the establishment of acetogens in the rumen. Better utilization of tree leaves and agro-industrial by-products through identification of antinutritional factor(s) degrading microbes and their establishment in the rumen may also be possible. Similarly, the genomic information of cattle and other ruminants could assist in identifying animals that are low methane emitters and have better feed conversion efficiency (Hegarty *et al.*, 2007). Potential applications of studies on farm animal genomes, including rumen microbial genomes, are innumerable.

The plant kingdom in the tropics is full of diversity. Tropical plants contain a large number of bioactive phytochemicals, the activity and diversity of which in tropical regions is considered greater than in temperate regions (Makkar, Francis and Becker, 2007). Local knowledge of using herbal products is also rich in many developing countries. With the ban on antibiotic growth promoters in the EU and increasing pressure on North American countries to follow suit, efforts are underway to identify natural plant growth promoters. The PCR and oligonucleotide probing methods for studying gut microbial ecology are affordable and within the capacity of molecular biology laboratories in developing countries. The application of these tools along with conventional tools could give an edge to developing countries over developed countries by identifying compounds from their rich and diverse flora that could be useful for the manipulation of rumen fermentation. They might, for example, be used to decrease methane emissions and increase the uptake of nitrogen and carbon by rumen microbes, and thus improve gut health while conserving the environment. The demand for natural products that enhance livestock productivity and animal welfare and make animal agriculture environmentally friendly will increase substantially in the future. The potential exists for developing countries to capture a large segment of the business in this area.

The use of enzymes and other additives in feeds, the development and use of genetically improved crops for animal feeds – including forages having higher water use efficiency, salt and drought tolerance, high quality, and low lignin; the development of animals with

high feed conversion efficiency through biotechnological means (e.g. MAS or cloning) and their widespread use would help mitigate problems linked both to global warming and land degradation. In addition, the biotechnologies discussed in this Chapter that improve animal health, fertility, productivity and efficiency would decrease greenhouse gas emissions by decreasing the number of animals needed to yield a given quantity of product. These are some examples, among many others, of the potential applications of biotechnologies in addressing the environmental impact of livestock production. It may be noted that strategies for mitigating greenhouse gases often also contribute to the adaptation of the livestock sector to climate change (FAO, 2009b).

### **Sustainable management of animal genetic resources**

The genetic diversity of livestock is in a state of decline globally. According to FAO (2007), 20 percent of the world's livestock breeds are at risk of extinction and the risk status of a further 36 percent cannot be determined owing to the absence of information. As mentioned previously, demand for increased production has led many countries to import exotic germplasm. Many livestock farmers have moved to cities to seek alternative livelihoods and left their livestock behind. Improved management of animal genetic resources is high on the agenda of most nations, and FAO is contributing enormously to this cause. Some developing countries, often in collaboration with international partners, are characterizing animal genetic resources using genetic markers and other conventional tools with the aim of gathering the information necessary to propose plans to conserve and utilize their resources more effectively. Molecular technologies may be a useful tool in determining the genetic basis for the adaptation of local breeds to their environment, including their ability to resist endemic diseases. Molecular genetics in concert with conventional breeding approaches can be used in the development of genetic improvement programmes for indigenous breeds, making them more competitive with exotic breeds and helping to ensure their *in situ* conservation while improving the livelihoods of their keepers. In some cases, breeds may risk extinction before utilization plans can be enacted and *in vitro* conservation will be a short-term solution. The development of new approaches for collecting and preserving germplasm, including improved cryopreservation methods, can contribute to achieving this objective. Advances in animal cloning technologies would be invaluable to increase the efficiency and decrease the costs of regenerating extinct populations from somatic cells and DNA which are relatively cheap to collect and store.

### 3.8 IDENTIFYING OPTIONS FOR DEVELOPING COUNTRIES

With reference to the stock-taking exercise that has been central to this Chapter, a number of specific options can be identified that should assist developing countries make informed decisions regarding the adoption of appropriate biotechnologies in the livestock sector in the future.

#### **Biotechnologies should build upon existing conventional technologies**

Solving new problems will require novel ideas and may involve new technologies. However, substantial impact of new biotechnologies can only be realized at the ground level in developing countries if the capabilities and infrastructure to effectively use conventional technologies are in place. For example, molecular diagnostics and recombinant vaccines will not improve the health or well-being of animals if an effective animal health infrastructure does not exist. Semen sexing and ET have no relevance in places where less advanced reproductive technologies such as AI are not well established and systems for the distribution of improved germplasm are not in place. The same is true for the application of MAS where animal identification and recording systems for relevant traits (e.g. milk yield, resistance to diseases, growth rate) are not in place. Efficient animal identification systems, e.g. based on ear tags, animal passports and computer recording, are needed in order to take full advantage of molecular markers, DNA sequencing and other advanced biotechnologies for animal genetics, nutrition and health. Similarly, biotechnology-based nutritional strategies will not work if farmers do not have access to adequate feed resources or to the knowledge of how to prepare balanced diets. An exception to this rule could be the use of simple “turn-key” approaches such as on-site “dip-stick tests” for disease diagnosis, provided these are low-cost and simple to use and interpret. This situation could be analogous to the use of mobile phones which has revolutionized communication in developing countries. “Dip-stick tests” have the potential to make a significant contribution to enhancing food security through the rapid diagnosis of diseases in remote areas. This would certainly make disease control and eradication programmes more effective and efficient.

In short, although biotechnologies have many advantages, they should not be considered as replacements of conventional (non-biotechnology) approaches just because of a desire to follow a scientific fashion. The introduction of a biotechnology should be done after assessing the field situation critically, considering the various options available and the comparative advantages and disadvantages of each in solving a specific problem, and the final decision should be made in a scientific and unbiased manner, remembering that technology *per se* is not a solution in itself.

## Biotechnologies should be integrated with other relevant components in any livestock development programme

Not all biotechnologies can be applied successfully in all situations at all times. Each biotechnology has relevance to a specific situation and in most cases it has to complement conventional technologies and other components of the livestock production and marketing system to elicit the desired impact for the farmer. An example is the integrated programme involving farmer organizations, extension workers, researchers and policy-makers that reversed the decline of a locally-adapted dairy sheep breed in Tunisia (Djemali *et al.*, 2009). This initiative was backed up by sound R&D involving biotechnologies such as AI and oestrus synchronization. These technologies were minor components but played a vital role in the success of the entire programme. They would not, however, have brought about the desired results had the other components not been in place. In other words, the focus should be on the reasons for the low food security and poor livelihoods of farmers rather than the solutions of applying a particular biotechnology. The importance of integrating biotechnologies as components rather than being the primary focus of a livestock development programme was illustrated clearly in Case Study 3.6.4, where AI was implemented as part of a wider programme to improve dairy production in Bangladesh.

The increasing importance of environmental issues also means that these should also be considered in any livestock development programme. For example, plans for the application of biotechnologies for nutrition (e.g. prebiotics and probiotics, enzymes and silage additives) should consider both the effects on animal productivity and the potential impacts (positive or negative) of the technology on the production system and the environment.

## Application of biotechnologies should be supported within the framework of a national livestock development programme

Developing countries must ensure that animal biotechnologies are deployed within the framework of national development programmes for the benefit of producers and consumers and not as stand-alone programmes. The models of biotechnology interventions in developing countries differ distinctly from those in developed countries. The biotechnologies that are simple and cost-effective are more likely to be successful in developing countries. To ensure the successful application of a biotechnology in the complex and diverse animal agriculture scenarios present in developing countries, not only does the mitigation of technical challenges need to be addressed but also, and probably more importantly, issues like management, logistics, technology transfer, human capacity, regulation and intellectual property. This is particularly the case when a technology is well developed in developed countries and yet relevant to the needs of developing countries.

Policy-makers in developing countries should be aware that there will be practical, financial and legal obstacles that will preclude the full-scale adoption of many livestock biotechnologies. In such instances, strategies for adoption and use must be based on realistic expectations. Many biotechnologies are biased with respect to scale, so that their application is only economically feasible in large enterprises. The building of infrastructure (laboratories, equipment etc.) will not be possible in every country, so that North-South, South-South and public-private partnerships will be required, meaning that countries may have to accept the loss of some autonomy in exchange for access to certain biotechnologies. In such cases, capacity building in developing countries should be directed at understanding the technology and financial investments involved and should emphasize adapting and using the technology to meet livestock development goals unique to the country, rather than replicating an entire system at the local level.

With the SIT, for example, there is a strong positive correlation between the research capabilities of in-country biotechnologists and the scale of its application in the field. The translation of research into commercial enterprises requires solid science, long-term resource commitments and extensive steps of validation to reach the thresholds of reproducibility and profitability. Therefore, strong scientific drive, vision and entrepreneurial skills are needed for contributing to progress in animal biotechnologies. The capacity to conduct research in biotechnology and develop products cannot just be “turned on”. It requires prior nurturing over many years with an adequate and uninterrupted provision of funds, which is possible only through strong commitment from science and policy managers in developing countries.

### **Access to biotechnological products by end users should be ensured**

An appropriate model for scaling up and packaging the technology should be integrated into the development and application of biotechnologies and biotechnological products, particularly for vaccines, diagnostics, probiotics, prebiotics and enzymes so that the products are not cost-prohibitive. It has to be borne in mind that the target end users of these biotechnologies in developing countries are normally resource-poor farmers with limited purchasing power. Without this scaled-up business approach/model, even good science and quality biotechnological products might not deliver desired impacts at the field level. In the business model, it is also imperative to consider the intellectual property issues which impinge on several aspects of biotechnology. For example, for manufacturing a recombinant vaccine, developing countries might find that the use of antigens, delivery mechanisms, adjuvants and the process are already patented and subject to intellectual property conditions. Equally important in the business model is the cost of registration of a product such as a vaccine, which could be very high or prohibitive. To illustrate this,

registration of the TickGUARD vaccine against *B. microplus* required several vaccination trials on approximately 18 000 cattle. This took a long time to complete and consumed huge resources (Willadsen, 2005).

The fostering of private-public partnerships – particularly in the areas of AI and associated reproductive biotechnologies, the production of biotechnological products such as amino acids, feed additives, vaccines and molecular diagnostics, and bioinformatics – is expected to enhance the pace of development in the animal agriculture sector and help contribute to meeting the UN Millennium Development Goals.

### 3.9 IDENTIFYING PRIORITIES FOR ACTION FOR THE INTERNATIONAL COMMUNITY

The international community (FAO, other UN organizations, NGOs, development and donor agencies) can play a key role in international cooperation and in supporting developing countries to implement appropriate biotechnologies for their needs in the future. Below is a set of Priorities for Action for the international community to enable it to play this role.

- International support should be provided to developing countries for completing surveys and characterizing livestock diversity, within which molecular evaluation of genetic diversity is an important component.
- International institutions should provide assistance to developing countries in framing animal breeding policies that consider both indigenous and exotic animal genetic resources, and help them strengthen their AI infrastructure and capabilities. Policies should be based upon existing national action plans for animal genetics resources.
- Assistance provided in the adoption of biotechnologies to increase the genetic merit for livestock productivity in developing countries should be complemented by the creation and maintenance of markets for the end products.
- In order to enhance the impact of assisted reproductive biotechnologies such as AI, semen sexing, IVF, ET and germplasm cryopreservation, national and international public-private research and technology transfer partnerships must be built and strengthened.
- Through the support of international organizations, national and multinational cryobanks for storing animal genetic resources should be established. The legal framework for regulating the use of animal genetic resources and operation of cryobanks needs to be formulated.
- The establishment of public-private partnerships for the development and production of animal nutrition products of biotechnology should be considered at both national and international levels to increase the uptake of the technologies.
- Diagnostic approaches involve both serological and newer molecular techniques. Provision of training in diagnostics, potentially including international training courses, should

be supported by both international organizations and the nations concerned and they should ensure that internationally recognized standards such as those published in the OIE Animal Health Code are implemented.

- Training programmes for establishing quality assurance methods such as those published by OIE allow continuous assessment of the assays used, and network programmes for validation of diagnostic methods should be organized by international funding agencies as the area of disease diagnostics is beset with problems of validation.
- Reference laboratories for conventional and newer technologies including biotechnologies provide useful services in the diagnostic and vaccine control areas and should work in collaboration with national veterinary services. The proper establishment of reference laboratories to implement international standards (e.g. standards approved by OIE or the International Organization for Standardization [ISO]) should be supported by international organizations through training, advice and political negotiations to secure sustainable funding. The exact role of any reference laboratory has to be defined from the beginning. National and regional acceptance and support is vital to sustaining them.
- The early and accurate detection and efficient monitoring and control of transboundary animal diseases, particularly zoonoses, are of great international interest. Therefore, international cooperation in the development, uptake and adaptation for use of the associated biotechnologies is essential.
- The international community should help developing countries to integrate animal biotechnologies within the context of national livestock development programmes and overall developmental needs. Furthermore, the formulation of programmes should be based on solving specific problems rather than imposing specific solutions to these problems. Initiatives that aim to reconstruct (or tailor) animal biotechnologies to specific needs and localities as part of a comprehensive and holistic solution to a given problem are important and need encouragement as well as tangible support.
- International and national institutions alike should identify ways of improving cooperation to address issues pertaining to animal biotechnology. Firm and committed North-South and South-South collaborative programmes and partnerships should be developed and fostered through the consistent and long-term provision of sufficient funds.
- Short-sighted worldwide research policies have neglected animal research in recent years. The amount spent by developing nations on animal research should be increased. The international donor agencies should also designate increased funds for R&D in the area of animal science in developing countries.
- International funding agencies should support the training of people to perform quality research. Research competence is a prerequisite for harnessing the benefits of animal biotechnologies. The training programmes should be directed at young scientists and

complemented with incentives (e.g. subsequent employment, research funding and networking opportunities) to encourage graduates to apply their training to addressing livestock production issues in their home countries.

- Support for capacity building must extend beyond training for the adoption of a specific biotechnology to include investment in improvement of higher education in general. Academic and professional institutions in developing countries must be strengthened so that they may provide an intellectual base on which to build an understanding of the problems that confront livestock production and determine which solutions (including biotechnologies) are best to address the problems.
- Public awareness of advanced animal biotechnologies such as animal cloning and genetic modification should be encouraged and enhanced by international organizations based on sound scientific evidence of the technologies' efficacy, safety, and costs and benefits in the context of a developing country.

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