“Potential of biotechnology to manage animal disease”

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Animal Biotechnology

Animal biotechnology encompasses a broad range of techniques for the genetic improvement of domesticated animal species including selective breeding, artificial insemination, cloning, and genetic engineering. Learn about both biomedical and agricultural applications of animal biotechnology and some of the science-based and ethical concerns that are engendered by certain applications.
Key unsolved problems in animal production where biotechnologies could be fundamental to their solution

“Continued population growth and urbanization, global warming, the globalization of trade and the ongoing intensification of livestock, 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, 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.”

Ortiz, Rodomiro. 2010. Agricultural Biotechnologies in Developing Countries: Options and Opportunities in Crops, Forestry, Livestock, Fisheries and Agro-Industry to Face the Challenges of Food Insecurity and Climate Change.
Convention on Biological Diversity: “**Biotechnology** is any technological application that uses biological systems, living organisms or derivatives thereof to make or modify products or processes for specific use.”

<table>
<thead>
<tr>
<th>Genetics/breeding</th>
<th>Nutrition</th>
<th>Health</th>
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<tbody>
<tr>
<td>Artificial insemination</td>
<td>Feed additives: Amino acids, enzymes &amp; probiotics</td>
<td>Conventional vaccines</td>
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<tr>
<td>Progesterone monitoring</td>
<td>Prebiotics</td>
<td>Recombinant vaccines</td>
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<td>Estrus synchronization</td>
<td>Silage additives (enzymes and microbial inoculants)</td>
<td>Sterile insect technique (SIT)</td>
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<td>Invito fertilization and embryo transfer</td>
<td>Monensin/ionophores</td>
<td>Molecular diagnostics</td>
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<td>Molecular markers</td>
<td>Single cell protein</td>
<td>Bioinformatics</td>
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<td>Cryopreservation</td>
<td>Solid state fermentation of lignocellulosics</td>
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<td>Semen and embryo sexing</td>
<td>Recombinant somatotropins</td>
<td>Green = +++ Potential for generating impact (time frame &lt;10 years)</td>
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<tr>
<td>Cloning</td>
<td>Molecular gut microbiology</td>
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<td>Transgenesis</td>
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Ortiz, Rodomiro. 2010. *Agricultural Biotechnologies in Developing Countries: Options and Opportunities in Crops, Forestry, Livestock, Fisheries and Agro-Industry to Face the Challenges of Food Insecurity and Climate Change.*
FIELD OF DEATH  Cattle carcasses littered a pasture in 1900 during a rinderpest epidemic – Photo: G. R. Thomson. A vaccine has since eradicated the disease from the planet.
Conventional vaccines

- The application of inactivated or live attenuated vaccines offers a cost-effective measure to control or even eradicate an infectious disease, as exemplified by eradication of rinderpest.
- During the last two decades these vaccines have played a more prominent role in enhancing livestock production in developing countries.
- The value of biotechnology-based products for use in animal health was US$ 2.8 billion in 2007 (OIE, 2007) and the contribution of veterinary vaccines to this global market was approximately 23%.
- During the last 3 decades there has been a shift from treatment of clinical illness to prophylactic disease prevention. *Diagnostics and vaccines are expected to generate more business compared to pharmaceuticals in the animal health sector in the future.*

Ortiz, Rodomiro. 2010. *Agricultural Biotechnologies in Developing Countries: Options and Opportunities in Crops, Forestry, Livestock, Fisheries and Agro-Industry to Face the Challenges of Food Insecurity and Climate Change.*
Recombinant vaccines

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
- differentiation 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 and so far their use in developing countries is negligible.

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Recombinant vaccines

- According to an 2005 OIE survey, 17% and 50% of African and Asian countries, respectively produce or use animal vaccines that are biotechnologically derived.
- Most of these countries are using vaccines produced in other countries.
- In Africa, only 1 country reported using DIVA vaccine.

<table>
<thead>
<tr>
<th>Application of biotechnology-derived animal vaccines in different parts of the world (adapted from MacKenzie, 2005)</th>
<th>Global (44)*</th>
<th>Africa (17)</th>
<th>Asia (50)</th>
<th>Middle East (50)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of countries producing or using biotechnology-derived vaccines in animals</td>
<td>40</td>
<td>4</td>
<td>7</td>
<td>1</td>
</tr>
<tr>
<td>Number of countries using viral-vectored vaccines which include antigen(s) from unrelated organisms</td>
<td>26</td>
<td>2</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Number of countries using bacterial vectored vaccines which include antigen(s) from unrelated organisms</td>
<td>16</td>
<td>1</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>Number of countries using vaccines which have deleted antigen(s) to differentiate infected from vaccinated animals (DIVA)</td>
<td>22</td>
<td>1</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Number of countries using vaccines that include recombinant proteins</td>
<td>26</td>
<td>0</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>Number of countries using DNA vaccines</td>
<td>6</td>
<td>0</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Number of countries using other product (undefined)</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

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

Sterile insect technique

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.

Ortiz, Rodomiro. 2010. *Agricultural Biotechnologies in Developing Countries: Options and Opportunities in Crops, Forestry, Livestock, Fisheries and Agro-Industry to Face the Challenges of Food Insecurity and Climate Change.*
Trypanosomiasis: Cycle of infection

• Trypanosomiasis is a disease caused by blood parasites of the genus *Trypanosoma* and 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 to 60 million people in Africa were exposed to the bite of tsetse flies, which can result in sleeping sickness.

• Recent estimates indicate over 60 million people living in some 250 locations are at risk of contracting the disease, and under 10,000 new cases were reported in 2009 according to WHO figures, which represents a huge decrease from the estimated 300,000 new cases in 1998. The disease has been recorded as occurring in 36 countries, all in sub-Saharan Africa.

• It is endemic in southeast Uganda and western Kenya, and killed more than 48,000 Africans in 2008.
Sterile insect technique (SIT)

- SIT is an important component of an area-wide integrated pest management (AW-IPM) approach for freeing areas under agricultural development from the tsetse and trypanosomiasis disease burden.
- SIT has also been 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).
- Both trypanosomiasis and myiasis result in high morbidity and mortality in livestock causing large economic losses.
- The removal of some populations of the insect vectors from particular infested areas or regions is expected to act as a catalyst for higher economic growth in several developing countries.

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## Molecular diagnostics

Some important diseases and biotechnology-based diagnostic techniques used in developing countries (Source, OIE, 2008).

<table>
<thead>
<tr>
<th>Disease</th>
<th>Diagnostic techniques</th>
</tr>
</thead>
<tbody>
<tr>
<td>Foot-and-mouth</td>
<td>ELISA; RT-PCR</td>
</tr>
<tr>
<td>Rift Valley fever</td>
<td>ELISA; RT-PCR</td>
</tr>
<tr>
<td>Avian influenza</td>
<td>RT-PCR</td>
</tr>
<tr>
<td>Peste des petits ruminants</td>
<td>Immunocapture ELISA; Counter immunoelectrophoresis; Agar gel immunodiffusion; RT-PCR</td>
</tr>
<tr>
<td>Contagious bovine pleuropneumonia</td>
<td>Competitive ELISA; PCR</td>
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</table>

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, the 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.
POINT-OF-NEED Detection Systems

- Qiagen was selected by UN Food and Agriculture Organization (FAO) and International Atomic Energy Agency (IAEA) to supply 50 molecular testing devices for use in Africa, Asia and South America.

- These QIAGEN ESE Quant Tube Scanner can analyze DNA or RNA from viruses or bacteria even in remote settings, which eliminates the need for time-consuming sample shipments to central laboratories.

- Qiagen started supplying the platforms in November 2010 as a 3 year pilot
  - Avian Flu (H5N1) in poultry,
  - Peste des Petits Ruminants (PPR) in sheep and goats
  - Contagious Bovine Pleuro-Pneumonia in cattle.
"We predict that the application of next-generation sequencing will soon be sufficiently fast, accurate and cheap to be used in routine clinical microbiology practice, where it could replace many complex current techniques with a single, more efficient workflow."


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Current approach

Diagnostic technology for the genomics era

The assay screens a sample for 12 pathogens and 18 antibiotic resistance markers in a single tube, combining the workflow and cost of a dozen assays into a comprehensive low cost solution.

Diagnosis by sequencing as an example:

Pathogenica’s sequencing analysis architecture generates a complete picture of the pathogens present in a sample in under 30 minutes and enables analysis of antibiotic resistance dissemination, infection spread between sites and individuals, or discrimination of hospital acquired from community acquired infections (i.e. DIVA).

http://www.pathogenica.com/

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Figure 1: Three Hour Workflow

- **Extract Nucleic Acid**: Total nucleic acid extraction with standard commercial kit
- **Interrogate**: Thousands of pathogens assayed simultaneously
- **Amplify**: Individually barcoded patient samples

Reactions performed by serial addition to a single tube, minimizing workflow, enabling automation and reducing the risk of cross-contamination.
Is there a place for this type of technology in veterinary diagnostic setting?

Highly multiplexed assay technology provides an effective means to:
• screen up to 48 samples for a dozen pathogens and drug resistance markers in one assay
• distinguish closely related pathogens in order to track transmission of infections in a hospital setting
• achieve single base resolution for the cost of a traditional PCR assay
• No need to culture – aerobic/anaerobic bacteria and viruses

Multiple pieces of information from single test
• Rapid identification of multiple pathogens
• Track outbreak transmission between patients
• Distinguish antibiotic resistance genotypes
• Confirmation of complex multi-species infections
• Improve clinical infection control and management
• Simple work flow allows fast, single shift turnaround
### Potential applications of NGS in the diagnostic microbiology laboratory

<table>
<thead>
<tr>
<th>Application</th>
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<tbody>
<tr>
<td>Outbreak investigations</td>
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<tr>
<td>Epidemiological typing</td>
</tr>
<tr>
<td>Characterizing resistance determinants</td>
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<tr>
<td>Unknown organism identification</td>
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<tr>
<td>Detection of known virulence factors in clinically severe disease (e.g. toxins)</td>
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</table>

### Current limitations

<table>
<thead>
<tr>
<th>Limitation</th>
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<tbody>
<tr>
<td>Cost of personal genome sequencing platforms</td>
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<tr>
<td>Speed of data analysis</td>
</tr>
<tr>
<td>Limited user-friendly <strong>bioinformatics</strong> platforms available for sequence assembly and data analysis</td>
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<tr>
<td>Education – poor knowledge of genomics and informatics amongst diagnostic microbiologists</td>
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</tbody>
</table>

### Limited reference sequence data for many species

<table>
<thead>
<tr>
<th>Limitation</th>
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<tbody>
<tr>
<td>Difficulties with DNA extraction directly from clinical samples</td>
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### Future directions

<table>
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<th>Direction</th>
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<tbody>
<tr>
<td>Routine sequencing of important nosocomial pathogens</td>
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<tr>
<td>Metagenomics for complex microbial communities</td>
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<tr>
<td>Discovery of new pathogens, resistance mechanisms</td>
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</tbody>
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## Current status of animal biotechnologies and factors influencing their applicability in developing countries - HEALTH

<table>
<thead>
<tr>
<th></th>
<th>Extent of use</th>
<th>Public and government acceptance</th>
<th>Current technical capability for using technology</th>
<th>Current technical capability for adopting or developing new technology</th>
<th>Infrastructure and materials and tools available for using technology</th>
<th>Relative cost</th>
<th>Skills required for application</th>
<th>Potential for generating impact (time frame &lt;10 years)</th>
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</thead>
<tbody>
<tr>
<td>Conventional vaccines</td>
<td>++</td>
<td>+++</td>
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<td>+</td>
<td>+</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
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<tr>
<td>Recombinant vaccines</td>
<td>0</td>
<td>+</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
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<tr>
<td>Sterile insect technique (SIT)</td>
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<td>+</td>
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<td>++</td>
<td>+++</td>
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<tr>
<td>Genetically engineered sterile insect</td>
<td>0</td>
<td>+</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>++</td>
<td>+++</td>
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<tr>
<td>Molecular diagnostics</td>
<td>++</td>
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<tr>
<td>Bioinformatics</td>
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<td>+</td>
<td>++</td>
<td>+++</td>
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</tr>
<tr>
<td>Genetically engineered animals</td>
<td>0</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+++</td>
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</table>
Sleeping sickness

- Trypanosomiasis is a disease caused by blood parasites of the genus *Trypanosoma* and transmitted in Africa by tsetse flies.
- 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 to 60 million people in Africa were exposed to the bite of tsetse flies, which can result in sleeping sickness.
- Recent estimates indicate over 60 million people living in some 250 locations are at risk of contracting the disease. The disease has been recorded as occurring in 36 countries, all in sub-Saharan Africa.
- It is endemic in southeast Uganda and western Kenya, and killed more than 48,000 Africans in 2008.
Sleeping sickness endemicity status and distribution of *T. b. gambiens* and *T. b. rhodesiense* in sub-Saharan Africa.
Sterile insect technique has been one tool in the fight

- SIT has 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 solutions 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% 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.
- 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 monkey.

Ortiz, Rodomiro. 2010. *Agricultural Biotechnologies in Developing Countries: Options and Opportunities in Crops, Forestry, Livestock, Fisheries and Agro-Industry to Face the Challenges of Food Insecurity and Climate Change.*
Several strategies are being developed to control vector-borne diseases using GE insects e.g. mosquitoes/dengue fever.

Both GE and non-GE mosquitoes have been developed that promote (drive) the spread of a less-fit mosquito with reduced ability to spread disease through the population. The non-GE mosquitoes are beyond the scope of the Cartagena Protocol. However, given that their biosafety implications are as serious as those for GE mosquitoes, further discussion is needed on how they should be regulated...

*Even-handed regulation will ensure that one strategy is not chosen over another purely for its immunity to onerous requirements.*


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Animal Biotechnology and Genomics Education
The Cartagena Protocol on Biosafety (CPB) to the Convention on Biological Diversity

As of 2011, 162 countries had ratified or acceded to the CPB. Conspicuously absent are Canada, the United States of America, and Australia.

- **Fundamental document of the UN to ensure the safe handling, transport and use of living modified organisms (LMOs) resulting from modern biotechnology that may have adverse effects on biological diversity, taking also into account risks to human health**

- LMO defined as an organism the genes or genetic material of which has been modified in a way that does not occur naturally through mating or natural recombination or both (i.e. GE)

- **Precautionary principle** “lack of scientific certainty due to insufficient relevant scientific information and knowledge regarding the extent of the potential adverse effects of a living modified organism on the conservation and sustainable use of biological diversity in the party of import, taking also into account risks to human health, shall not prevent that party from taking a decisions as appropriate, with regard to the import of the living modified organism in order to avoid or minimize such potential adverse risks.”

- It does **not** allow the consideration of benefits and looks only at the risks associated with living modified organisms (LMOs).
Trypanosomiasis is a disease caused by blood parasites of the genus *Trypanosoma* and 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 to 60 million people in Africa were exposed to the bite of tsetse flies, which can result in sleeping sickness.

Until the transgenic cows come home: Researchers hope to engineer cattle like these in northern Uganda to resist the sleeping sickness parasite.

[http://www.genomics.liv.ac.uk/tryps/Key_Papers/PuttingSleepingSicknessToBed.pdf](http://www.genomics.liv.ac.uk/tryps/Key_Papers/PuttingSleepingSicknessToBed.pdf)
$2 million grant from the Bill & Melinda Gates Foundation and the US National Science Foundation to develop trypanosomiasis-resistant ApoL1 GE cattle.

Yet, even if the project pans out, there might not be much demand for an engineered cow, notes Sue Welburn, a molecular epidemiologist at the University of Edinburgh, UK. In the rural communities of Uganda, where Welburn works as part of a public-private partnership, “people are reluctant to accept anything transgenic,” she says.

Welburn acknowledges that cows are important—her research shows that the movement of infected livestock may be responsible for the spread of the disease from endemic to nonendemic regions in Uganda. But she argues that introducing transgenic cattle is neither the smartest nor the most cost-effective way to tackle the disease, noting that “we have a very effective way of getting rid of those parasites in domestic livestock. You just have to give them an injection of a trypanocidal drug. That’s quite a lot easier than proposing transgenic animals for Africa.”

http://www.genomics.liv.ac.uk/tryps/Key_Papers/PuttingSleepingSicknessToBed.pdf
Merging values and technology

All agriculture production practices impact the environment and can have adverse effects on biosafety, and the conservation and sustainable use of biological diversity.

Regulatory frameworks should formally evaluate the reasonable and unique risks associated with the use of GE animals (and other biotechnologies) in agricultural systems, and weigh them against those associated with existing conventional systems, and those of inaction (i.e. postponing a regulatory decision). Perhaps more importantly these risks have to be weighed against the benefits. This would represent a shift away from an uneven risk-assessment process that emphasizes ever diminishing marginal hazards, to one that includes a risk:benefit analysis to more objectively evaluate and communicate the likely impacts of approving a new GE organism.
Technologies based on modern biotechnology offer enormous potential for the production of vaccines, medicinal products and other veterinary products.

- The development and use of these technologies is currently concentrated to a few countries, while in others they are still not in widespread use.

- There is a need to extend this information and provide training in those technologies for which suitable development conditions exist.

- It will be necessary to foster the establishment of a comprehensive and effective regulatory framework for the safe use of these biotechnologies.

- Even-handed regulation based on the product (and not the process that was used to create it) will be required to ensure that one strategy is not chosen over another purely for its immunity to onerous requirements.

- The best regulatory approach will be one that allows new technologies to be used while preventing new risks to human health or the environment.

Funding for this project is provided by the National Institute of Food and Agriculture.

• Jim Womack, PD
• Alan Dabney
• Scott Dindot
• Noah Cohen

• Laurel Gershwin
• Terry Lehenbauer
• Cassandra Tucker
• Alison Van Eenennaam

• Jerry Taylor

• Chris Seabury
• Lawrence Falconer
• Lauren Skow
• Gary Snowder

• Milt Thomas
• Mark Enns

• Mike MacNeil
• Curt Van Tassell

• Holly Neibergs
• Shannon Neibergs

• Robert Hagevoort
• Tim Ross

OTHER COLLABORATORS
• Daniel Pomp (NC)
• Shiela McGuirk (WI)
• Adroaldo Zanella (Norway)
Our goal is to integrate research, education, and extension activities to develop cost-effective genomic and management approaches to reduce the incidence of BRD in beef and dairy cattle

Dr. Jim Womack, Texas A&M University, College Station, TX

The objective of this multi-institutional project is to reduce the incidence of bovine respiratory disease by:

• Capitalizing on recent advances in genomics to enable novel genetic approaches to select for disease-resistant cattle
• Developing improved DNA-based tests for disease diagnosis
• Providing educational opportunities for undergraduate, graduate and veterinary students to generate a future human resource for the continued reduction in bovine respiratory disease incidence
• Producing and delivering a variety of educational materials for beef and dairy cattle producers, and feedlot personnel on best management practices to reduce disease incidence

Agriculture and Food Research Initiative Competitive Grant no. 2011-68004-30367

Van Eenennaam NIAA 4/17/2013
Thanks for inviting me!

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US Bovine Respiratory Disease Coordinated Agricultural Project
http://www.brdcomplex.org

The “Integrated Program for Reducing Bovine Respiratory Disease Complex (BRDC) in Beef and Dairy Cattle” Coordinated Agricultural Project is supported by Agriculture and Food Research Initiative Competitive Grant no. 2011-68004-30367 from the USDA National Institute of Food and Agriculture.