73rd General Session International Committee Appendix
World organisation for animal health Paris, 22-27 May 2005

73 SG/10

Original: English

APPLICATIONS OF GENETIC ENGINEERING FOR LIVESTOCK AND BIOTECHNOLOGY PRODUCTS

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Summary: *The influence that biotechnology is having and will have in the future on animal health is now being realised well beyond the researchers' laboratories. An examination of the evolution of reproductive biotechnologies is presented. The latest techniques allow for the use of transgenic farm animals as sources of biologically activated proteins, bio-pharmaceuticals, as donors in xenotransplantations and for further research in gene therapy, all of which are important applications in human medicine. The beneficial applications of embryo transfer include disease control, transboundary movement of livestock and the provision of sexed sperm and sexed embryos. Although cloning of livestock is a multi-step complex process and the technology needs further evaluation, benefits such as the multiplication of desired traits and the conservation of animal germplasm clearly are substantial benefits. With the application of transgenesis in livestock, the benefits of disease resistance, improved meat, milk and wool quality and protein production in milk and meat (biofarm animals) are major benefits. It is predicted that biotechnology-derived vaccines will become common in animal health programmes where they can be shown to have improved efficacy and safety compared to conventional products.*

When carrying out risk assessments for genetically engineered animals, conventional techniques and tools will be useful but it is important to be aware that because limited data are available, the actual hazard identification will be a considerable challenge. As the new technologies with their adherent applications evolve, standard setters and regulators will be faced with the challenge of moving in parallel with technological advances.

In response to a questionnaire sent to Delegates of OIE Member Countries, only 40% of respondents indicated that their animal health regulatory administrations have the capability of conducting risk assessments on biotechnology derived animals or products. Likewise, 20% of respondents do not consider the guidelines for risk analysis adequate to help carry out an import risk analysis on biotechnology-derived animals or products. Furthermore, 50% of respondents do not have a regulatory framework in place to govern cloning, transgenic production or products of biotechnology such as vaccines. Public perception in relation to cloning and biotechnology-derived animals will present considerable challenge to Member Countries with 79% reporting no public support for cloning and 52% reporting biotechnology-derived animals perceived as controversial.

There is considerable work that must be initiated by both Member Countries and the OIE to allow appropriate progression in the very important field of biotechnology and animal health.

1. Introduction

The continued challenge that OIE Member Countries currently face with the globalisation of agriculture is not limited to the animal health issues arising from infectious and zoonotic diseases. The fact that reproductive biotechnologies combined with genetic manipulations have developed animals with varying traits poses a new and different kind of challenge to the OIE and the scientific community. We are entering an era which will see more widespread use of reproductive technologies combined with gene manipulations to develop livestock and aquatic animals that can propagate superior or desired traits. Central to the idea of genetic improvement of domestic animals by selective breeding or cross breeding, the world today is presented with the scientific opportunity through assisted reproductive technologies and genetic manipulations that could have a positive impact on public health and well being.

The techniques of molecular biology provide a great potential for the production of important medical and agricultural products. The production of biotechnology-derived drugs and vaccines relies heavily on recombinant DNA technology for the design of useful products. The production of recombinant proteins in 'transgenic animal bioreactors' is another application of modern biotechnology in agriculture. Preceding the release of any biotechnology-derived animal into the environment, extensive safety evaluations are carried out to verify if it is safe according to currently available expertise. However, as the risk assessments rarely evaluate the risk as zero, biotechnology products in agriculture are regulated, and the purpose of the existing safety guidelines is to assure that these products pose a minimal risk to public health and the environment. The purpose of this OIE Technical Item II is to discuss the technology that is used to develop biotechnology-derived animals, the applications in the fields of animal health and diagnostics, the delicate balance between regulatory frameworks and the ethics of the science, sound risk assessments based on current science and to present to the Member Countries an overview of the applications of biotechnology in the global context.

2. Techniques

The past four decades have witnessed the commercial adaptation of four generations of reproductive biotechnologies, particularly with cattle. It started with artificial insemination and moved to a second level when *in-vivo*-derived embryos were harvested and transferred. The third generation consisted of the *in-vitro* fertilised embryos, sex sorting sperm, ovum pick-up and cloning and finally we are at a stage when we are using functional deletion and addition of specific genes to the offspring's genome through transgenesis, combined with powerful molecular biology techniques of siRNA (small interfering RNAs) and the use of viral vectors. Recently there have been combinations of transgenics being propagated by somatic cell nuclear transfer techniques, thus taking reproductive biotechnologies a step further.

Embryo transfer of *in-vivo*-derived embryos was a huge step to increase the propagation of germplasm of the desired trait. Compounded by the success of multiple ovulation and embryo transfer technology (MOET) there was an upsurge in the transfer of bovine embryos, especially in North America, where 35% of the 538, 312 embryos available worldwide were transferred in 2002 (7). The *in-vitro* embryo production system was instrumental in bringing a spurt in embryo transfer activities across the globe. The data collected in 2002 showed that more than 80,000 *in-vitro* produced (IVP) embryos were transferred. The IVP embryos provided a window of opportunity for 'invasiveness' in terms of techniques to assess the embryo characteristics. This included obtaining an embryo biopsy for the purpose of prenatal sex determination. The use of biopsies for pre-natal genetic diagnosis, blastomere assessment for cloning and for other purposes is still utilised in many settings.

Production of identical offspring with somatic cell nuclear transfer (cloning) was a big step forward, as earlier embryos were split prior to transfer to achieve the same result. As early as the 1980s Steve Willadsen showed that embryonic cells from 8-16 cell embryos could be used for embryonic cell cloning, but the birth of 'Dolly' in 1996 proved that there was a possibility of reprogramming adult differentiated cells. Nuclear cloning is, however, currently an inefficient process with a success rate of 6-10% of embryos transferred to the cattle resulting in a healthy offspring. There are concerns related to higher losses in pregnancy, placental dysfunctions, incorrect epigenetic reprogramming and post natal complications. These abnormalities may be

epigenetic errors that can be corrected during gametogenesis. While the technology is still not beyond the infancy stage, the confidence among the researchers that this could result in genetic preservation and disseminating genetic gains, begs attention both from the scientific point of view as well as other issues related to ethics, welfare and product safety. In contrast to the cloned animal in which some of the anomalies are viewed, the offspring of clones produced following sexual reproduction appear phenotypically normal. Because nuclear cloning can also result in physiologically normal animals, it is anticipated that the initial commercialisation of this technology will focus on producing small numbers of high value animals for breeding purposes, and possibly also transgenic dairy animals capable of producing valuable pharmaceuticals in their milk.

The first transgenic livestock were produced almost 20 years ago by micro-injection of DNA into the pronuclei of zygotes. Pronuclear microinjection of DNA was the standard method for producing transgenic animals until now. However, this is now being replaced by more efficient methods based on somatic nuclear transfer, which also permit targeted genetic modifications. Lentiviral vectors and siRNA technology are also being used for transgenesis. Research has been focussing on chimera generations via injection of pleuripotent cells in early pre-implantation embryos or blastocysts. Transgenesis has also been achieved in livestock by the culture of spermatogonia and their transplantation into recipient males. A novel approach is the use of active siRNA, and the simplicity of this has facilitated adoption of this method to generate permanent or transient knockouts for specific genes. The combination of siRNA and lentiviral vector technology may provide enhanced gene transfer efficiency and specificity in gene knockouts for cattle. Transgenic farm animals are important in human medicine as sources of biologically active proteins, bio-pharmaceuticals, as donors in xenotransplantation, and for research in gene therapy.

3. Applications

For ease of explanation, this part will deal with the applications of technologies in current use that have gained importance in the last three or four decades. The first consideration is the embryo transfer technology which is still the backbone of any assisted reproductive biotechnology. The embryo may be produced *in-vitro*, and may be manipulated or cloned, yet it still has to be transferred to a recipient to bear the offspring.

3.1. Embryo transfer: *in-vivo* and *in-vitro* embryos

The embryo transfer industry grew rapidly in the late 1970s, both in terms of the number of practitioners and in the number of donors flushed. North America has continued to be the centre of commercial embryo transfer activity with more than 190,000 bovine embryos transferred annually. The importance of follicle wave dynamics and methods for the synchronisation of follicular wave emergence has simplified the means by which superovulation might be achieved, resulting in increased embryo production per unit time. Some of the applications that are of clear benefit to the embryo industry are:

3.1.1. Disease control

Several large studies have now shown that the bovine embryo does not transmit infectious diseases, if recommended precautions such as those mentioned in the OIE *Terrestrial Animal Health Code* (the *Terrestrial Code*) are followed. In fact the International Embryo Transfer Society (IETS) – an international embryo biotechnologists association, has categorised disease agents based on the risk of transmission by a bovine embryo. It is noteworthy that none of the infectious diseases studied have been transmitted by *in-vivo*-produced bovine embryos, provided embryo handling procedures were done correctly. Consequently, it has been suggested that embryo transfer could be used to salvage genetics in the face of a disease outbreak, which could also be a useful option in the establishment of disease free herds.

3.1.2. Transboundary movement of livestock

The intercontinental transport of live animals costs a lot, whereas an entire herd can be transported, in the form of frozen embryos, for much less. Added benefits of frozen embryos over live animals include reduced risk of disease transmission, reduced quarantine costs, a wider genetic base, the retention of genetics within the exporting country, and adaptation. The procedures recommended by the IETS for embryo handling that have been endorsed by the OIE, greatly reduce the possibility of infectious agents being transferred in *in-vivo* derived embryos. However, there is still some risk of infectious agent transmission with *in-vitro* derived, abattoir retrieved oocytes, and those with zona breaching.

The development of effective methods of freezing embryos has made embryo transfer a much more efficient technology, that is no longer dependent on the immediate availability of suitable recipients. Researchers have directly addressed the question of using IVP as a substitute for *in-vivo* production of embryos by conventional embryo transfer procedures. However, it is unclear whether IVP is a realistic alternative to conventional superovulation and embryo transfer for production of embryos from reproductively healthy cattle.

3.1.3. Sexed sperm and sexed embryos

Determination of the sex of pre-implantation bovine embryos with the use of the polymerase chain reaction (PCR) is also commonly employed in some situations. However, the removal of the biopsy from the embryo requires a high level of operator skill, and embryo biopsy is an invasive technique that results in the invasion of the integrity of the zona pellucida, and results in some reduction in the viability of the embryo. The flow cytometric technology used to separate X- and Y-bearing sperm into live fractions has been improved over the last 10 years. With a purity of 90%, about 10 million live sperm of each sex can be sorted per hour. In both cases there is a potential to establish embryo banks to obtain the progeny of choice in any setting. This can reduce the unnecessary cost of producing large number of embryos and transfers related to that.

The science and practice of artificial embryo production (*in-vitro*-produced and *in-vivo* derived) has given us insight into the early embryonic period and the later fetal and neonatal development. By studying in greater detail the aberrant features of artificially produced embryos, one may find that these very same mechanisms lie behind the so-called "normal" fetal and neonatal loss. This will not only help to improve the efficiency but also provide us with the possibility of critically analysing the more invasive procedures (like biopsy, pronuclear injections etc.) that precede the embryo transfer.

4. Nuclear transfer cloning

Cloning livestock following somatic cell nuclear transfer involves multiple steps, each with potential for disturbing development of the embryo and foetus, and affecting health during adulthood. For these reasons, the technology needs to be thoroughly evaluated to fully appreciate the longer-term consequences on the animals produced. However the application of this technology, although limited at the present time, shows promise of increased benefits:

4.1. Multiplication of desired traits

Cloning could enable the rapid dissemination of superior genotypes from nucleus breeding flocks and herds, directly to commercial farmers. Genotypes could be provided that are ideally suited for specific product characteristics, disease resistance, or environmental conditions.

4.2. Conservation of animal germplasm

Cloning technology can help salvage the germplasm of indigenous species that are near extinction, including intra-species nuclear transfer procedures which can be used to rescue genes from endangered species.

4.3. Research model

Animals can be cloned for research purposes, to provide a basic research model of genetically identical individuals, reducing variability in the outcome of experiments.

4.4. In association with transgenic applications

Cloning can provide a rapid way to increase the population or number of the transgenic animals, and this can permit the testing of genetic stability with reduced progeny intervals.

An increasing body of international data indicates that the major abnormalities in clones are probably epigenetic in nature and do not appear to be transmitted to offspring, even when male and female clones are mated. However, there is the need for molecular confirmation of this observation, which will be important in providing confidence in large scale breeding applications of genetically elite cloned livestock. Despite the present limitations of cloning, milk or meat from these cloned livestock does not appear to be materially different from conventionally bred animals (1). If the acceptability and utility of this emerging technology are to be improved, it is important to understand the biology behind nuclear cloning to improve the health and viability of the cloned animals produced and their surrogate mothers.

5. Transgenesis

Application of Transgenesis in livestock has been instrumental in the development of animals that are: resistant to diseases, have improved meat, milk or wool quality, can increase proteins in their milk or meat (biopharm animals), or which have characteristics which are environmentally friendly. The production of recombinant proteins is one of the major successes of biotechnology. Milk, egg white, blood, urine, and seminal plasma can be sources for recombinant proteins. Numerous experiments have shown that the prediction of the expression of transgenic proteins is possible to a limited extent. The purification of proteins from the milk or other body fluids is not too difficult except for those present in blood. The available techniques to produce pharmaceutical proteins can be used to add nutriceuticals to milk, and to improve carcass quality or meat composition of the livestock or farm animals. Antibodies seem to be the kind of proteins that will be most frequently used, as they could be a good alternative to antibiotics for some infectious diseases.

The application of techniques related to the production of recombinant proteins in milk has reached a certain degree of maturity but there is much to do yet. The combination of recent advancements in reproductive technologies with tools of molecular biology opens the horizon to a new era in transgenic biotechnology. The growing amount of data from the human genome project will certainly inspire intense genome sequencing in livestock, and somatic cloning will pave the way for the introduction of novel transgenics in livestock.

6. Xenotransplantation

The possibility that xenotransplantation may offer an opportunity to have an alternative to organ transplantation from human donors is very attractive to researchers. The application of transgenic technologies can alter donor animals, so that the stimulus to induce immune rejection in recipient patients is much reduced. The research in this area is focussed on the pig genome, to make organs and tissues more compatible to humans. Disruption of the gene causing hyperacute rejection response (Galactose alpha 1, 3 galactose), by gene targeting in GM pigs, has been achieved and further use of nuclear transfer for propagating them is in progress. This provides immense hope for patients awaiting transplantation and needing organs or tissues to fight major medical conditions such as heart disease and diabetes.

7. Vaccines

There are three major considerations in the registration of vaccines for use in animals, and biotechnology-derived vaccines have both advantages and disadvantages in each of these areas. The OIE *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals* (the *Terrestrial Manual*) and the *Terrestrial Code* (5) make mention of these conditions. However, considerations are described in different ways in different regulations, but the key elements that must be demonstrated in any vaccine are efficacy (not worthless, satisfactory potency), purity (not contaminated) and safety (not dangerous or harmful to the environment, humans or animals).

Efficacy is usually determined following a challenge of vaccinated and control animals using a specific disease model. The observed protection from clinical disease or death following a specific dose and route of vaccination is used to formulate a claim for the vaccine product. Subsequent batches or serials of vaccine are compared to the original batch used in this study, and a potency test is developed to enable the prediction that each serial with a satisfactory potency test will lead to the same level of protection observed in vaccinated animals in the study. Biotechnology-derived vaccines which incorporate antigens which are known to be targets of a protective immune response and which present those antigens in a manner to maximise the required type of immune protection may enhance efficacy. These vaccines may also have more thermostable antigens, perhaps plant-derived proteins, allowing this vaccine potency to be maintained in the absence of a cold chain in tropical countries. In many disease models, though, the use of live attenuated disease organisms in vaccination leads to the highest level of immune protection, even if there are some residual safety concerns.

Purity is determined by ensuring that the organisms and ingredients used to make the vaccine are not contaminated with other microorganisms, or toxins, or perhaps prions. With many biotechnology-derived vaccines, the platform expressing the antigens of interest is well characterised, and the vaccine can often be produced with a minimal risk of contamination. Growing more conventional pathogens for the production of killed vaccines often requires the use of materials of animal origin in order to maximise the expression of the antigens of interest, which increases risks of contamination of the vaccine. On the other hand, plant-made vaccines grown in open fields risk having undefined contamination such as weeds, fungus or insects, but this may not impact on the safety of the product if administered orally.

Safety is probably the most important potential advantage of biotechnology-derived vaccines. DNA vaccines which do not require oil-based adjuvants are safer to administer by humans and are also safer for animals including fish than some conventional vaccines. Vectors for recombinant vaccines can be selected to minimise any possibility of reversion to virulence which sometime occurs with live attenuated vaccines. Conventional vaccines may also contain toxic elements that are not completely inactivated or removed by the manufacturing process, and which may not be necessary for stimulating immune protection in the animal. Removal of these toxins in biotechnology-derived vaccines improves product safety to both humans handling the vaccine or eating vaccinated animals, and to the animals themselves. The use of genetically-modified vectors or plants in the environment does raise some concerns of environmental safety which may be different than the use of conventional live attenuated vaccines. Steps must be taken in the approval process of these products to evaluate and to minimise all of the potential concerns which can be identified.

Ultimately, the usefulness of vaccines will be determined by their availability. This, in turn, is affected by elements such as cost of production and acceptance of a role of vaccination in disease control programmes. Biotechnology-derived vaccines will become common in animal health programmes in cases where they can be shown to have improved efficacy or safety when compared to conventional products, and if they are available.

8. Risk assessment considerations

Advances in genetic engineering continue to emerge at an accelerating pace, enhancing the potential for its applications. It is anticipated that commercialisation of farm animals genetically engineered to produce unique traits will soon be a reality. Both transgenic and cloned animals raise potentially new concerns about food safety, human health, animal health (and welfare), and the environment. Therefore there is a pressing requirement to develop methodologies to adequately assess the safety of such animals.

To this end, there is a need to bring together scientists, regulators, international organisations, such as OIE, FAO and WHO and other stakeholders to identify and review the science-based data and concerns relevant to science-based risk assessment and management of genetically engineered animals released into the environment. These expert consultations could help design research to solve problems as well as to identify and develop appropriate management practices to minimise risks associated with genetically-engineered animals.

Risk assessment for genetically engineered animals is not much different than that for conventional animals but because of limited data, general uncertainties and unknowns, the paramount point of a risk assessment, hazard identification, remains an enormous challenge for the risk assessors.

The major difference resides in the identification of hazards from potential genetic abnormalities (phenotypically or genotypically) that can be of possible harm. Since animals exhibiting grossly undesirable effects are likely to be eliminated during commercial development, the areas of concern are those caused by subtle dysregulation of genes.

The following questions, arising from these subtle genetic abnormalities, will render risk assessment more complex and probably, in early stages, result in a high degree of uncertainty:

- How would one detect them?
- How would subtle genetic hazards from genetically-engineered animals differ from the gene dysregulation arising in conventional animals?
- How frequent do they occur compared to conventional animals?
- Would these genetic hazards pose a risk?
- Should they pose a risk, how to measure risks if they happen?

In addition to risk assessment, risk analyses pertaining to genetically-engineered animals involves risk communication (throughout the entire process) and risk management. These other components of the analysis must also take economical, ethical and societal as well as animal welfare factors into account.

At this early stage of the development of genetically-engineered animals, assessments will be based upon data extrapolated from related studies done with other genetic modifications or in other species, often utilising material supplied by the companies marketing the products. In this context, Governments must maintain the public trust at a high level through impartiality, integrity and transparency of its decision making process with respect to genetically-engineered animals and their products.

It is perhaps reasonable to believe that in the coming years, the analysis of the risks associated with genetically-engineered animals will become routine, as it has for the import of conventional animals and animal products. Ongoing improvements in the techniques of genetically-engineered animal production will likely reduce the incidence of animal health problems now recognised, and the continued growth of the body of knowledge will reduce the uncertainties that now exist. Additionally new techniques and increased experience will also improve methods of risk management.

Conversely, future research and new techniques will perhaps identify hitherto unknown problems for the risk analyst to deal with - as old issues get resolved, new ones may emerge. It is safe to assume that the challenges presented by animal biotechnology for the risk analyst and other regulatory staff will continue for some time to come.

9. Regulatory Framework

New technologies need to be controlled by guidelines or regulations so as to maximise benefits and minimise risks to humans, animals and the environment. The acceptance of agricultural biotechnology will depend on whether consumers see an obvious personal and societal benefit in the new products. However, the role of the regulators is also to assist the public make an informed decision by critically evaluating the data related to the technology, and determining the level of risk to the consumer, the animal population, and the environment. Development of legislation and the regulatory provisions do not move as quickly as advances in science. In most cases the regulations are developed to address the concerns of the consumers and society and to provide a much needed level of protection.

Since transgenesis and cloning are relatively new scientific techniques, transgenic animals are new organisms for which there is limited information. The issues associated with the regulation and biosafety of transgenic animals pertain to environmental impact, food safety, animal health and welfare, trade and ethics. To regulate this new and powerful technology predicated on limited background information is a challenge not only for the regulators, but also for the developers of such animals, who strive to prove that the animals are safe and merit bio-equivalency to their conventional counterparts. In principle, an effective regulatory sieve should permit safe products, while forming a formidable barrier for those assessed as posing an unacceptable risk.

The regulation of products derived from biotechnology can be based on the principles used for conventionally produced animals. Regulations and standards for determining a responsible use of animal biotechnology in food and agriculture are based on principles that take into account criteria such as benefits and risks, scientific basis of biotechnology and effects on the environment, and must also consider animal welfare and social acceptance (4). Transgenic animals may be viewed as most acceptable if the end result of the genetic manipulation applied is to provide better quality of life for humans, or to provide 'environmentally friendly' alternatives to 'factory farms'. The regulations that each country employs to safeguard the public, the animal population, and the environment from unintended effects of novel products or technologies are specific to the way the regulatory framework is established. For example, some countries may have an approach to regulate the technology, while others may be regulating the products of biotechnology. Some of the salient considerations for sound regulation developments are:

- high standards for human and animal health and welfare
- development of clear standards and guidelines for assessments
- provision of sound scientific basis to evaluate associated risks
- consultation and involvement of stakeholders in the development of regulations
- maintenance of genetic diversity and conservation of environment
- building upon existing regulations

The existing scenario allows us to extrapolate the standards and to develop regulations from the continued research and work being done by international organisations such as the OIE, FAO and the International Embryo transfer Society (IETS). IETS was founded in 1974 with 82 charter members, representing researchers, academics and veterinary practitioners. A growing majority of the IETS membership is composed of basic researchers representing government, industrial or academic institutions, including human medicine. However, the IETS has played a very important role in the dissemination of basic and applied information, allowing for the rapid growth of the embryo transfer industry in the 1980s and 1990s. In particular, the Import/Export Committee of the IETS now referred to as the Health and Safety Advisory Committee (HASAC) has been instrumental in gathering and disseminating scientific information on the potential for disease control by the use of bovine embryo transfer. There was the round table meeting on sanitary issues related to embryo transfers between the IETS and the World Organisation for Animal Health (OIE) in 1985 (*Rev. sci. tech. int. Epiz.*, 1985, **4**, 843-913), resulting in the drafting of sanitary procedures for the international movement of embryos in the OIE *Terrestrial Code*, and the International Embryo Movement Symposium, sponsored by the IETS. These events along with continued close collaboration between the IETS

and the OIE have made the international movement of cattle embryos possible. In this regard, The Manual of the International Embryo Transfer Society *"A procedural guide and general information for the use of embryo transfer technology emphasising sanitary procedures"* (6) has become the reference source for sanitary procedures used in export protocols. Today most of the international movement of embryos are based on the recommendations of IETS many of which are endorsed by the OIE *Terrestrial Code* and the procedures documented in the Manual of IETS are the red book letters for the regulators.

International organisations such as FAO have conducted workshops as technology has progressed, including "Gene-based technologies" (2), and the expert consultation on genetically modified animals (Rome 2003). The recommendations of these consultations and workshops form a solid basis for development of regulations in general. The forums where the food safety of cloned animals and international movement, identification and traceability of the embryos is discussed and standards recommended, takes place under the auspices of different subcommittees of IETS.

In essence the regulatory framework may be very specific to the region and the country, yet the consideration is more towards harmonising the approach, so as to facilitate sharing of safety information, and to help countries prevent the spread of disease or infections through germplasm. The OIE has therefore an important role to play as a standard-setting body in accordance with its mandate under the WTO-SPS Agreement.

10. Questionnaire

The OIE sent a questionnaire to the Delegates of all 167 OIE Member Countries to assemble baseline information on some questions relating to applications of biotechnology for livestock and animal health products. Responses were received from 91 countries, including a broad cross-section of Member Countries from all regions. The results are presented as Appendices I and II and are summarised in this preliminary analysis. The questions and tabulated summary results will be posted on the OIE website. This information will provide a useful baseline to identify topics for discussion in international reference groups and standard setting bodies such as the World Organisation for Animal Health (OIE), and international organisations such as Veterinary International Cooperation for Harmonisation (VICH), and International Embryo Transfer Society (IETS).

The responses to this survey have illustrated a number of common interests and concerns for animal health regulatory agencies as well as livestock producers and consumers. There are many potential opportunities for international collaboration in establishing technical standards and risk assessment procedures for these technologies. It is clear that the OIE and affiliated standard setting bodies will have a key role to play in facilitating the dissemination of information on development of appropriate risk-based regulatory standards, approval processes, and certification procedures for biotechnology-derived livestock and animal health products.

The OIE formed a Biotechnology Working Group in 1989. This Working Group was active until November 2000, however it is no longer functioning, and it has been decided that rather than maintaining an ongoing working group for this topic, the work will now be incorporated into other Ad hoc Groups which will be assigned to study specific topics. It is possible that this survey and the review papers in the Scientific and Technical Review will help to identify issues for further discussion, including some topics which might be referred to *ad hoc* groups for in depth analysis and recommendations.

The following 91 countries provided a response to the questionnaire: Algeria, Andorra, Angola, Argentina, Australia, Austria, Azerbaijan, Belgium, Benin, Bhutan, Bosnia and Herzegovina, Brazil, Brunei, Burkina Faso, Cambodia, Canada, Colombia, Congo (Dem. Rep.), Costa Rica, Cote d'Ivoire, Cyprus, Czech Republic, Denmark, Dominican Republic, Ecuador, Egypt, El Salvador, Eritrea, Estonia, Finland, France, Georgia, Germany, Ghana, Greece, Guatemala, Guinea Bissau, Hungary, Iceland, India, Japan, Kazakhstan, Kenya, Kuwait, Latvia, Lithuania, Luxemburg, Madagascar, Mali, Mauritania, Mauritius, Mexico, Moldavia, Morocco, Myanmar, Namibia, Nepal, Netherlands, New Caledonia, New Zealand, Nicaragua, Norway, Pakistan, Paraguay, Peru, Philippines, Poland, Portugal, Romania, Serbia and Montenegro, Slovakia, Slovenia, Spain, Sudan, Swaziland, Sweden, Switzerland, Taipei China, Tanzania, Thailand, Togo, Trinidad and Tobago, Tunisia, Turkey, Uganda, Ukraine, United Kingdom, United States of America, Uruguay, Venezuela and Zimbabwe.

11. Definitions

Eighty-one of the ninety-one OIE Member Countries (89%) responding to question 1 agreed with the proposed definitions as applicable to livestock biotechnology. Agreement was consistently high across all geographical areas. Where respondents did not agree with the definitions, the most highly suggested sources for definitions were the Cartegena Protocol on Biosafety to the Convention on Biodiversity and Codex Alimentarius in its Principles for the Risk Analysis of Foods Derived from Modern Biotechnology (CAC/GL 44-2003).

12. Risk Analysis

Fifty-three of the eighty-nine (60%) Member Countries that responded to question 3 reported that the animal health authority in their country does not have the capability to conduct risk analysis on biotechnology-derived livestock and biotechnology products. The two main reasons reported for not performing risk analysis on these commodities are the absence of training (53%) and the lack of knowledge (26%).

Eighty-four per cent (75 of 89) of the respondents indicated that they do not have a dedicated unit conducting risk analyses pertaining to biotechnology commodities (question 4). Risk analyses are being conducted by the epidemiology and surveillance unit in 29% of cases, and 37% of respondents answered that other units conduct these risk analyses. Only 9% use an external consultant to conduct a risk analysis on these commodities. Forty five per cent of the Asian respondents indicated that risk analyses are conducted by the Import-Export unit.

In question 5, the major factors identified by the animal health authorities as being considered when determining the risk associated with these biotechnology commodities are respectively: food safety (26%), animal health (26%) and environmental impact (23%).

Only 25% of the Member Countries have conducted (or received a request to conduct) a risk analysis on biotechnology commodities, for a total of 33 requests (question 6). In 52% of the cases (17 cases out of 33), the risk analyses were conducted (or were requested to be conducted) on biotechnology products, whereas only three were requested on cloned animals and seven were requested on transgenic animals. Most of the requests are from countries in the Americas (13 cases).

Only 29% (25 out 89 respondents) of the Member Countries are willing to make their risk analysis document available for peer review or for public consultation, using official government publication as the main means of dissemination. Peer reviews are mainly conducted internally (45%) within the veterinary services. Only European Member States reported having risk analyses peer reviewed internally (37%) or externally (36%) at approximately the same proportion.

Seventy-seven per cent of the respondents (63 of 81) to question 8 considered that the "Guidelines for risk analysis" contained in the OIE *Terrestrial Code* were adequate to carry out an import risk analysis on a biotechnology commodity. Forty-four per cent of the Member Countries (8 of 18 respondents) that considered that these "Guidelines for risk analysis" were not adequate are from the Americas, and these Member Countries also reported receiving most of the requests (39%) for conducting a risk analysis (question 6).

13. Regulatory framework

Sixty-four per cent (58 of 91) responding OIE Member Countries to question 9 reported that they did not produce biotechnology-derived animals or biotechnology-derived products for use on animals. Out of the 31 Member Countries responding "yes" to question 9 (note that 2 did not answer), 14 countries (45%) are European Member States.

Respondents to question 10 reported having capabilities in the following fields: cloning (17%), transgenic production (20%) and products of biotechnology for use in animals such as vaccines and/or drugs (28%). Thirty-five per cent of respondents to the questionnaire did not provide an answer to this question.

Approximately half of OIE Member Countries responding to question 11 reported having a regulatory framework in place to govern the use of a biotechnology commodity (44 reported having a framework out of 89 respondents). Sixty-two per cent of European Members States and fifty-three per cent of Countries from the Americas responding to question 11 indicated that they have a framework in place to govern the use of such commodities

14. Research

Slightly less than half (47%) of the 91 responding OIE Member Countries to question 12 reported that there is research being conducted in their country into biotechnology-derived animals and products, including vaccines and drugs. At 64%, Asia had the highest percentage of responding countries engaged in animal biotechnology research activities, followed closely by Europe at 59%, the Middle East at 50%, the Americas at 41%, and Africa at 25%. Although this question covers a wide variety of activities, the results indicate that this is an active area of research.

15. Animal Vaccines

Forty out of eighty-nine responding OIE Member Countries (44%) to question 13 reported that they produced or used animal vaccines in their countries that are biotechnology-derived. This may include experimental products that are not currently licensed for general use, since the question did not ask that countries specify the licensing or marketing authorisation status of products.

Twenty-six of the forty countries who replied said that they produced or used viral vectored vaccines (29% of the responders to this question) which included antigen(s) from unrelated organisms. Sixteen countries (18%) reported using bacterial vectored vaccines which include antigen(s) from unrelated organisms. Twenty-two countries (25%) reported using vaccines which have deleted antigen(s) to differentiate infected animals from vaccinates (DIVA). Twenty-six (29%) of countries produced or used vaccines which included recombinant proteins, and six countries reported using DNA vaccines (7%). One other biotechnology-derived vaccine was reported but not described in the questionnaire.

Eighty-seven OIE Member Countries responded to question 14 which asked how biotechnology-derived vaccines and/or drugs are generally perceived by the public in their countries. Twelve countries (14% of responders) indicated they were perceived as safe, twenty-five (28%) said they were controversial, and thirty-nine countries (45%) said that the public was mostly unaware of biotechnology-derived vaccines. Eleven other countries (13%) made a variety of other comments in response to this question.

When examining public opinion research done in individual countries, it seems unlikely that approximately half of the population in the responding countries are truly "aware" of biotechnology-derived veterinary vaccines and drugs. People can be unaware of products but, when asked, think the products are safe and well-regulated. They can also be aware and think the products are not safe and aren't well-regulated. These questions are often separated in detailed polling for that reason.

16. Technologies

Sixty-six per cent of the Member Countries that responded indicated that they do not have livestock cloning and/or transgenic animal production facilities in their country. However the European Region indicated that 50% of the 34 countries that responded (69%) do have livestock cloning and/or transgenic animal production facilities in there country.

Sixty-four out of ninety-one countries (72%) indicated that biotechnology-derived animals or their products are not permitted in the food or feed supply in there country. In the Asian Region the 43% indicated that biotechnology-derived animals or their products are permitted in the food or feed supply in there country.

Eighty-three countries (83 of 91) responded to question 17. 79% of the responders indicated that there was no public support for cloning of animals. Of the 12% that indicated there was public support for cloning of animals the main purpose chosen was for the rescue of endangered species and generating stem cells. (36% each)

When Member Countries were asked if there are transgenic animals present in their country, out of the 54% that replied to the questionnaire 79% indicated that there were no transgenic animals in their country. Of the 24% that indicated there were transgenic animals in that country, 45% reported that the animals were generated for the purpose of biopharmaceuticals.

Of the 89 Member Countries that responded to this question, 56% indicated that their country does not have the laboratory capacity to identify and detect transgenes in the food/feed supply. However, in the Asian Region 50% and the European Region 65% indicated that they do have the laboratory capacity to identify and detect transgenes in the food/feed supply.

17. Public perception

Like question 14 above, this question asked people what they thought about the way other people think. Overall the evidence suggests that as with most applications, people employ the same case-by-case risk/benefit analysis when evaluating these new techniques. In public opinion research on biotechnology, there is a clear hierarchy of support for various applications, and often health applications are at the top of the list, and food applications are nearer to the bottom.

For questions 14 and 20 that asked about public perception, it is very difficult to meaningfully compare data from different public opinion questionnaires (i.e. different questions asked under different circumstances). These questions measure the perception of government workers about public opinion (as opposed to actually measuring public opinion). There are some efforts to create agreed-upon methodology and questions (e.g. by international groups of academics and researchers) and it could be useful if OIE could be informed of the expertise of these people.

18. Conclusion

In conclusion the OIE may wish to consider further work as follows:

1) Development of a definition for Biotechnology which can be agreed by OIE Member Countries.

2) Development of standards and guidelines for research on containment and environmental release of live attenuated vaccines in animal health.

3) Development of recommendations and guidelines for use of DNA vaccines in food animals.

4) Development of guidelines and recommendations for somatic cell nuclear transfer cloning – guidance for interspecies cloning, recognising that this process has the potential to increase the possibility for transmission of diseases between species.

5) Develop objective criteria for assessing the health of embryos and animals derived from cloning, and associated safety of cloned livestock and their products.

6) Develop policy guidelines for exclusion of unapproved animals and products from the livestock population, and segregation from the feed and food supply.

7) Develop identification, testing, and certification guidelines for international trade in livestock animals and their products for which biotechnology procedures have been employed to confer disease resistance.

8) Incorporate standards into relevant OIE documentation such as the *Terrestrial Manual* and the *Terrestrial Code*, as well as the companion standards for aquatic animals.

9) Development of guidelines relevant to the application of Nanoscience/Nanotechnology as it relates to animal health.

Acknowledgement:

I wish to express my sincere appreciation to Drs Gifford, Kochhar, Hutchings and Moreau for the technical expertise they provided in the preparation of the paper. I also wish to thank Mr Sacha Gagnon for the questionnaire layout, data entry and analysis.

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