REDUCING THE RISK OF TRANSBOUNDARY ANIMAL DISEASES THROUGH NUCLEAR TECHNOLOGIES

A. Introduction

The challenge of ensuring food security for a world population that will grow to over eight billion people in the next 20 years can be met, in part, by assisting smallholder farmers in developing countries to improve the utilization of locally available land, water, and plant resources to intensify and increase animal production and productivity. This will require not only more sustainable livestock production, but also more efficient approaches, tools, and strategies for preventing, diagnosing and controlling animal diseases. The amount of available animal protein for human consumption is already limited, but the fragile food security situation is further exacerbated by increased movement of animals and animal products due to expanding world trade and the growing effects of climate change that can result in changes in the geographical distribution of pathogens and their vectors. Resource-poor developing countries will become increasingly vulnerable to emergencies caused by the growing prevalence of infectious diseases, especially transboundary animal diseases (TADs). A complicating factor is that more than 60% of the TADs are zoonotic diseases (i.e. diseases of animal origin that infect humans), such as Human Immunodefficiency Virus (HIV), H5N1 (Avian Influenza) and H1N1 (Swine Flu), Rabies, Rift Valley Fever, and Trypanosomosis.

Classical or traditional techniques for diagnosing threatening diseases are well in place, but often lack the sensitivity and specificity needed to make accurate and timely diagnoses of diseases. Nuclear and nuclear related technologies have these features and are therefore increasingly being used to complement traditional diagnostic and tracing technologies to improve the early and rapid diagnosis and control of animal diseases through tracing and vaccination strategies [II-1]. The IAEA, through the development and application of nuclear and nuclear-related technologies, is at the forefront of developing and validating early and rapid diagnostic techniques that are simple to use, inexpensive and can be applied in a "laboratory limited" environment, such as those located in rural and decentralized areas; in the tracing of diseases through the application of stable isotope techniques; and in the application of irradiation technologies to provide safe and user friendly vaccines.

The application of nuclear technologies, in combination with conventional technologies, has contributed to concrete improvements in the number, condition and health of animals resulting in improved livelihoods for millions of people worldwide. For example, it is estimated that the eradication of rinderpest saves Africa more than 1 billion USD per year (FAO). The unique characteristics of nuclear technologies not only contribute to our efforts to reduce transboundary animal disease risks, but also to the tracing and monitoring of animal movements (e.g. the tracing of disease infected migratory birds), as well as to the timely and proactive control and prevention of diseases through the use of vaccines.

B. Nuclear and Nuclear-Related Techniques for Disease Diagnosis

Nuclear applications have driven modern biotechnological research by providing more sensitive, specific and cost effective diagnostic platforms or assays to detect and characterize the disease pathogens [II-1]. Many of these nuclear based applications are being used in Member States for diagnosis of TADs such as rinderpest and rabies. The use of nuclear technologies allows the detection and characterization of pathogens within 24 hours of their onset, helping to differentiate one particular virus strain from another [II-2]. An example of this differentiation is noted in the case of the Influenza A H1N1 virus, from Influenza A H5N1. Nuclear techniques are also important in determining the nucleic acid sequence that describes the capacity of a particular virus strain to cause a disease. Different strains of the

same virus may affect birds and also humans e.g Influenza A H5N1 low pathogenicity versus Influenza A H5N1 high pathogenicity. (Fig. II-1) [II-3]. The latter causes deaths in more than 60% of infected humans. The isotopic analysis of the genetic make-up of such a virus can be used by health authorities in making decisions ranging from public notification – as was the case of Influenza A H1N1 (low pathogen) - to immediate pandemic action in the case of Influenza A H1N1 (high pathogen) [II-4]. This information not only aids disease control personnel and policy makers in their attempts to control and eliminate veterinary and public health pathogens, but also forms the basis for decision-making that affects transboundary trade and travel.

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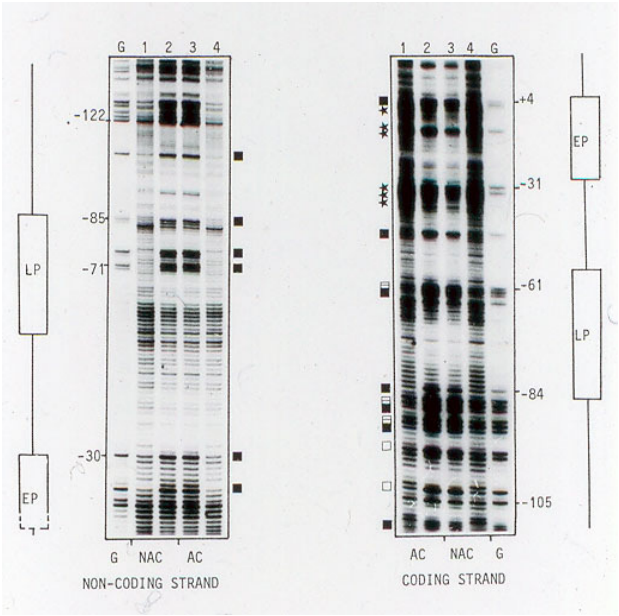

FIG. II-1. Phosphor-32 labelled protein-DNA analysis to study the operational control of active and non-active pathogenic genes to determine why certain pathogens are more aggressive than others. Nucleic acid sequence differences were observed in the Late Promoter (LP) and Early Promoter (EP) regions of the RNA transcription responsible genes of different Avian Influenza strains

Radioisotope-labelled assays that use isotope levels that are below the limit of disposal are under development. Isotope-based nucleic acid hybridization approaches are used to detect genetic material in host tissues that will allow direct identification of infected animals as well as provide information of epidemiological importance in relation to the strain type or variant of the agent. These tests depend on the preparation of suitable DNA probes labelled with sulphur-35 or phosphor-32 and their amplification *in vitro* by a nucleic acid amplification technique (PCR) to increase the amount of the specific target.

Nucleic acid thermal amplification technologies shorten the time for a test result to less than a day and in many cases a result can be obtained within an hour [II-1]. Recent successes using this technology include the development of tests to diagnose diseases such as the Peste des Petit Ruminants disease and capripox virus disease (the collective word for goatpox, sheeppox and cattlepox viruses) and in the sequencing of the different genomes. To set up an appropriate control against the outbreak of one of the three poxviruses in a livestock herd, the outbreak virus needs to be identified. Currently, the capripox virus family, although closely related, requires three different vaccines for protection, i.e. there is no cross-protection between the different capripox virus strains. Sheeppox virus, goatpox virus and cattlepox or lumpy skin disease virus, the third member of the capripox virus genus (Fig. II-2) can be

differentiated using the nuclear related thermal amplification real-time PCR approach, thereby selecting the correct vaccine to protect against the homologous pathogen [II-5].

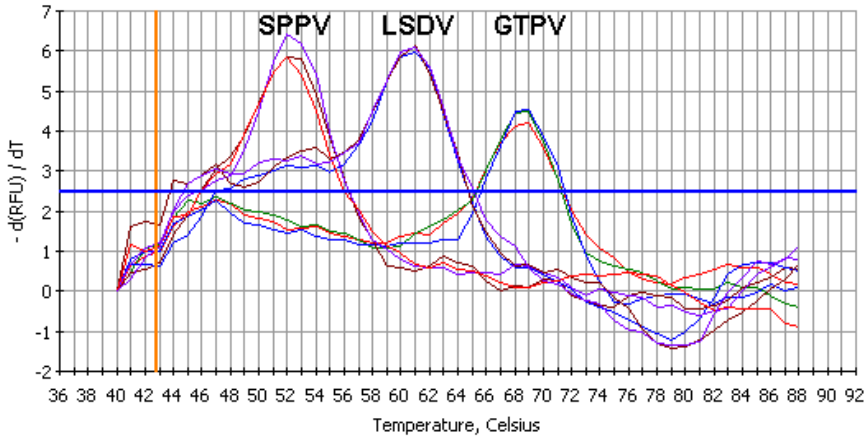

FIG. II-2. Discrimination of sheeppox virus, cattlepox or lumpy skin disease virus and goatpox virus based on their genetic sequence differences is possible using molecular DNA thermal amplification technologies. The Y-axis indicates the signal amplitude and the X-axis the temperature in degrees celsius.

Nuclear technologies are also vital to animal disease diagnosis where rapid decision-making would be an advantage, and especially in situations where the suspected disease occurs in difficult to reach or remote areas that are far from the laboratory [II-1]. The time saved by determining whether a disease is present or not, could be the difference between containing a disease at its point of origin and protecting human lives or preventing the spread of a disease to an animal market place or further afield. Conventional molecular techniques including thermal amplification or PCR require sophisticated, expensive equipment (Fig. II-3). A robust test at the molecular level, i.e. the loop mediated isothermal amplification (LAMP) PCR, has been developed using nuclear techniques, which is a more cost effective alternative to thermal DNA amplification. The LAMP PCR can be carried out within 30 to 60 minutes in a simple water bath at constant temperature and the presence or absence of the isothermally amplified DNA product can be detected visually, i.e. a change in color (Fig. II-4). Another advantage of the LAMP PCR platform is that it can be developed for use on-site or on farm as a penside (point of care) rapid diagnostic test [II-1].

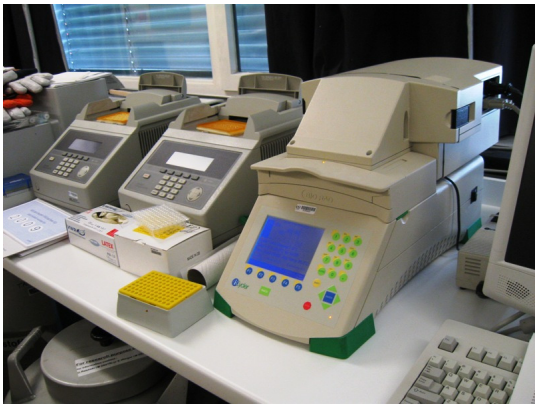

FIG. II-3. Different models of thermal DNA amplification cyclers (PCR Machines). Isothermal DNA amplification technologies will reduce our reliance on this expensive equipment.

FIG. II-4. Visible color changes in reaction tubes allow discrimination of positive and negative results when using the isothermal DNA amplification or LAMP PCR for diagnosing avian influenza.

C. Migratory Connectivity: Using Stable Isotope Analysis to Determine the Role that Wild Birds Play in Disease Outbreaks

A unique use of nuclear techniques is the ability to trace wild birds in order to determine if and whether they may contribute to the spread of the Bird Flu. Highly Pathogenic Avian Influenza (HPAI - Influenza A, H5N1 Bird Flu) causes disease and death in wild birds and poultry, and can also affect humans. HPAI outbreaks have resulted in losses of hundreds of millions of birds and caused serious economic damage to the poultry industry worldwide. In addition, Bird Flu is a zoonotic disease with a high mortality in humans and consequently has led to the death of several hundred people. Historically, similar influenza epidemics have killed millions of people, and the threat of a pandemic disease caused by Bird Flu today, makes it one of the most important animal and human health hazards currently facing humanity [II-3]. There is evidence that wild birds can be infected with Bird Flu and it is possible that migratory wild fowl could play a role in its dissemination (Fig. II-5).

FIG. II-5. The origins and flight-path of migrating bar-headed geese can be established by using stable isotope analysis of flight feathers.

Given the potential for wild birds to spread Bird Flu, more information is required about their movement. Millions of birds fly each year to and from over-wintering sites and a more concerted effort is required to investigate the poorly known routes of migrant birds in Africa, the Americas, Asia-Pacific, Central Asia and Europe. An ideal approach is to use a non-

invasive stable isotope analysis (SIA), to establish the origin and flight-path of a migratory bird [II-6, II-7].

Stable isotopes are currently used for tracing food origin. They provide a unique signature to a specific location, based on the availability of the isotope, which is also incorporated into animal products [II-6]. Their signature composition is dependant on the soil, water and plant chemical composition of each location. This feed and water signature is unique to each location and can be traced in the deposits (e.g. feathers) of the birds [II-7]. A small number of natural isotopes are involved in important biological and ecological processes. They are measured by mass spectrometry to determine isotopic differences relative to international standards and reported as ratios in delta (δ) units as parts per thousand. Of most interest are the hydrogen (δD) ratios found in metabolically inert, seasonally grown tissues, such as feathers and claws that accurately reflect the ratios in lakes, rivers and oceans and in groundwater in the migratory path of the birds. The isotopic signatures of a few individuals are representative of an entire population, hence any of the individuals from that population can provide information on movement. Feathers retain this information until replaced or moulted, which typically occurs only once per year. If the isotope profile of a particular bird population is known, any individuals from that population can provide information on the global migration of that species [II-8].

The hydrogen isotope composition of potable water varies spatially across the globe but global grids of hydrogen water isotopes have been constructed that can then be compared to animal samples of known or unknown origin. These grids are constructed using the data from the IAEA's Global Network for Isotopes in Precipitation (GNIP). Collecting isotope data from feathers of migratory bird species will reveal migration patterns; enable identification of the breeding areas of birds sampled in intermediate stopover sites; and in samples collected from disease outbreak sites, might provide greater understanding of the role that wild birds play as carriers of disease [II-9]. Currently, measurements of stable isotopes are done using costly isotope ratio mass spectrometry (IRMS) systems that require a well-equipped laboratory. However, newly introduced analyzers (Fig. II-6) with near infrared laser technology are small, transportable and require low maintenance, making it more affordable to measure isotopes. There are currently no conventional techniques which allow this kind of tracing of diseases.

FIG. II-6. A low cost answer to isotope ratio mass spectrometry (IRMS). This stable water isotope analyzer uses an infrared laser for measurement.

D. Radiation Inactivation: the Future "Gold Standard" in Vaccine Development

Vaccination is a cost-effective way of preventing and controlling disease. Although anti-viral and anti-bacterial vaccine development has been successful, there are few vaccines for parasitic diseases because of the risk of further infection by active parasites in the vaccine. The inactivation of pathogens via irradiation is promising because it is a reliable method of applying a safe vaccine - 100% inactivated - against pathogenic diseases [II-10]. Their

potency has been tested and success has been achieved with the advent of the first human radiation-attenuated anti-parasite vaccine for malaria. For many pathogens, a relatively low dose of gamma irradiation from a cobalt-60 source is sufficient to inactivate the organism, e.g. malaria irradiation at 150 Rad, *Fasciola* irradiation at 30 Gy, *Brucella* irradiation at 6kGy, while viral pathogens require higher doses e.g. RVF irradiation at 25kGy.

This opens a new approach to immunization, especially when dealing with problematic diseases, like Rift Valley Fever and various helminth (parasitic worms) and protozoal (unicellular parasites) diseases [II-11, II-12]. There is a considerable body of evidence to suggest that radiation-attenuated or radiation-inactivated vaccines are safer as well as a more effective and feasible "gold standard" for vaccine efficacy. Conventional alternative vaccines, such as recombinant vaccines, have not yet lived up to their promise to achieve comparable and effective levels of protection as those provided by irradiated vaccines.

Diseases caused by the liver fluke parasite *Fasciola spp* are important due to their worldwide negative economic impact and zoonotic nature; an irradiated vaccine is technically feasible. *Schistosoma bovis* and *S. japonicum* present additional targets for radiation attenuated vaccines for livestock [II-10]. The life cycles of all of these parasites are similar, involving snail intermediate hosts that produce the infective stages, which could be target immunogens for vaccine development. Studies in Sudan with *F. gigantica* have shown promise in protecting cattle, and demonstrate the technical feasibility to adapt the process to field application. The basic parameters of radiation dose, numbers of parasites, immunization route and numbers of immunization doses have already been established. Efforts can now be concentrated on developing pilot manufacturing techniques. Similar promising results have been obtained with *S. japonicum* - an important zoonosis in China, though diseases caused by pathogenic animal trypanosomes affect livestock productivity in other parts of Asia, Africa, and South America. Parasite attenuation requires optimization of the radiation dose to generate metabolically active, non-replicating parasites that are able to promote immune responses in skin and draining lymph nodes without leading to parasite invasion of the bloodstream.

Gamma irradiation inactivates viruses but leaves the viral proteins in their native conformation thereby greatly enhancing their immunogenicity and efficacy. The reduction in effective virus particles (or its titre – measure of concentration) due to inactivation would be less for irradiation than for chemical treatment (i.e. the irradiated viruses look like native viruses but can not cause disease) and therefore should elicit a more potent immune response [II-10, II-11, II-12]. This could impact the cost of manufacture and result in dose-sparing in relation to virus production. In addition, flow-through methods of irradiation rather than batch processing will need to be developed for viral inactivation or attenuation. It would also enable easier monitoring of the inactivation process by means of *in vitro* culture. For some viral vaccines where there is poor protection, i.e. where the vaccine is not eliciting a protection, [II-10, II-11] and the safety margin is small, i.e. where the vaccine is not safe for use and needs to be administrated together with an antibiotic agent, e.g. bovine babesiosis [II-12], irradiation will lead to safer and more cost effective products. Irradiation would also speed up the production processes and be useful in dealing with emergency situations as well as in providing better quality control and assurance in terms of standardisation and immunogenicity of vaccine strains.

In addition, it is foreseen that gamma irradiation will increasingly be used in the preparation of pathogenic viruses for use as antigen in diagnostic tests like RVF Immunoglobulin G and Immunoglobulin M assays, to ensure innocuity of biological material or in the preparation of diagnostic kits to extend their shelf life.

E. Conclusion

The world continues to demand more and healthier animals and animal products that are environmentally safe, clean and ethical. This demand poses far-reaching challenges for

animal scientists on the critically important need to improve technologies in animal production and health in order to ensure food security, poverty alleviation and environmental protection on a global scale.

Nuclear applications drive modern biotechnological research by providing more sensitive, specific and cost effective diagnostic platforms or assays to detect and characterize disease pathogens. The application of nuclear technologies - in combination with conventional technologies - contributes to improvements in the number, condition and health of animals resulting in improved livelihoods of millions of people worldwide.

Alongside advances in animal health, nuclear applications in animal reproduction and breeding show promise in assisting smallholder farmers worldwide in ensuring sustainable livestock production. The much anticipated revelation of the first bovine genome sequence and map, published in **Science** in April 2009, is a step forward in improving and utilizing animal genetic resources. With important input from scientists at the IAEA, cattle genome sequencing will contribute to the understanding and utilization of indigenous breeds to improve worldwide livestock productivity.

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