

Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture and FAO/IAEA Agriculture and Biotechnology Laboratory, Seibersdorf

Animal Production & Health Newsletter

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To Our Readers

Dear Colleagues,

The biggest event this year was undoubtedly, the successful International Symposium on Sustainable Improvement of Animal Production and Health that was held from 8 to 11 June 2009, here in Vienna. It was attended by more than 400 participants from about 100 Member States of the IAEA and FAO, including several international organizations, with oral and poster contributions. The most important aspects of the symposium were to renew old and form new acquaintances, to discuss common topics and strategies and to form networks and partnerships to address animal production and health problems. The symposium was indeed topical and designed to address issues of importance to our Member States. The new and emerging areas of interest such as One Heath, Food Security and Safety and our ability to produce more and healthier animals and animal products in an 'environmentally safe, clean and ethical' way were hotly discussed.

The five Sessions at the Symposium were:

- Interactions among nutrition, reproduction and genotype
- Effects of nutrition, reproduction and environmental factors on animal productivity
- Transboundary, emerging and zoonotic diseases
- One-health
- Achieving food safety and security in the 21st century



Some of the conclusions and challenges that animal scientists face, whose primary concern have been improving livestock productivity, are more extensively reported on in this newsletter. In addition, we will publish full length papers of all the oral presentations, and some of the most imminent poster presentations, as symposium proceedings shortly.

Both past and future activities are described in further detail in this newsletter and are also accessible at our website (http://www-naweb.iaea.org/nafa/aph/index.html). Please contact us if you have any further ideas, comments, concerns or questions. Concerning news from the subprogramme, we have to say goodbye to John Crowther who is retiring after an illustrious scientific career of more than 40 years. I do not have to go into details about his scientific achievements or his charismatic personality, but as an animal health specialist, John was singlehandedly responsible for the introduction and adaptation of the nuclear based applications of ELISA platforms to our FAO and IAEA Member States. As serological detection and surveillance tool, there is no other technology that can rival this technology. He was a valuable teammember since 1995 and I want to thank him for his contributions and friendship and want to wish him and Giovanna only the best for the future. We will remain in close contact and will continue to make use of his expertise. On a sunnier note, I want to welcome Adama Diallo to the subprogramme. Adama's return to the subprogramme as head of the Animal Production Unit at our Seibersdorf laboratory is most opportune and timely and welcomed by all. His scientific standing, animal health experience and extended international relations and connections are essential components to enhance our delivery to our Member States even more. In addition, I want to welcome Len Dimailig, Violeta Dicusara, Christian Schwarz, and Francisco Berguido, to the subprogramme; Len as temporary secretary and project assistant, Violeta and Christian as genetic breeding interns and Francisco Berguido as peste-des-petits-ruminants and immunology consultant. We want to welcome all as members of the subprogramme and wish them a pleasant and productive time with us.

Gerrit Viljoen, Head, Animal Production and Health Section

Staff

Joint FAO/IAEA Programme of Nuclear Techniques in Food and Agriculture,

Headquarters: Vienna International Centre, Wagramer Strasse 5, P.O. Box 100, A-1400 Vienna, Austria; Telephone: (+43 1) 2600+ Extension; Fax (43-1) 26007;

Email: Official.Mail@iaea.org

Name	Title	email	Extension
Qu LIANG	Director, NAFA	Q.Liang@iaea.org	21610
Gerrit J. VILJOEN	Molecular biologist,	G.J.Viljoen@iaea.org	26053
	Head, Animal Production & Health Section (APHS)		
Mario GARCIA PODESTA	Consultant, Animal Reproduction, APH	M.Garcia-Podesta@iaea.org	26048
Antony LUCKINS	Consultant, Animal Health, APH	A.Luckins@iaea.org	26054
E. Nicholas ODONGO	Livestock Nutritionist, APH	E.Odongo@iaea.org	26845
Kathrin SCHATEN	Junior Professional Officer, APH	K.M.Schaten@iaea.org	21618
Hermann UNGER	Veterinarian, APH	H.Unger@iaea.org	21644
Svetlana PIEDRA CORDERO	Project Assistant, APH	S.Piedra-Cordero@iaea.org	26051
Anna Lyn DIMAILIG	Project Assistant, APH	A.Dimailig@iaea.org	26052/21679
Roswitha SCHELLANDER	Project Assistant, APH	R.Schellander@iaea.org	26052

FAO/IAEA Agriculture and Biotechnology Laboratory, A-2444 Seibersdorf, Austria

Name	Title	email	Extension
Andrew CANNAVAN	Analytical Chemist, Head, Agrochemical Unit	A.Cannavan@iaea.org	28395
Adama DIALLO	Head, Animal Production Unit (APU)	A.Diallo@iaea.org	28355
Caroline Melanie ADOMBI	Biochemist (Consultant), APU	C.M.Adombi@iaea.org	28380/28323
Francisco BERGUIDO	Immunologist (Consultant), APU	F.Berguido@iaea.org	28356
Sanne Charles BODJO	Molecular Biologist (Consultant), APU	S.Bodjo@iaea.org	28222/28333
Jolanta KOLODZIEJEK	Biochemist (Consultant), APU	J. Kolodziejek@iaea.org	28385
Charles Euloge LAMIEN	Biochemist (Consultant), APU	C.Lamien@iaea.org	28379
Mamadou LELENTA	Veterinarian, Laboratory Technician, APU	M.Lelenta@iaea.org	28321
Massoud MALEK	Geneticist, APU	M.Malek@iaea.org	28358
Eva-Maria WINGER	Parasitologist, Laboratory Technician, APU	E.M.Winger@iaea.org	28303/28424
Kyoko MAKOVICKY	Clerk, APU	K.Makovicky@iaea.org	28362



The Animal Production Unit, Seibersdorf, is a collaborating Centre for ELISA and molecular technologies in animal disease diagnosis for the OIE.

Forthcoming Events

Regional (AFRA) Training Course on Molecular Techniques (RAF5057)

Technical Officer: Hermann Unger

The meeting will be held from 22 to 26 June 2009 in Botswana.

The purpose of the training course is to provide comprehensive and up-to-date theoretical and practical information on the nuclear and nuclear related molecular diagnosis of transboundary animal diseases. The course is intensive, but is designed to establish basic understanding of fundamental molecular biological principles and biotechnological applications, including guidelines and hands-on training on practical aspects of PCR, good laboratory practices and requirements for setting-up PCR laboratories, standardization of PCR protocols, the implementation of PCR diagnostic tests and good service delivery, awareness of difficulties as well as advantages of the assay, current advances in the PCR procedures and the placement of PCR in relation to other tests.

Consultants Meeting on the Socio-Economic Impact of Disease Prevention

Technical Officer: Kathrin Schaten

The meeting will be held in Aug/Sept 2009 in Vienna, Austria.

In the field of animal health there are many proven, efficacious answers to disease control, however, understanding how beneficial these strategies are in the overall context of increasing animal productivity requires detailed analysis of their implementation. The objective of this meeting is to evaluate methods to measure the socio-economic impact of prevention, surveillance and the early and rapid diagnosis of disease compared to the post-epidemic action and therapeutics used to provide us with a clearer understanding of the value and sustainability of disease control interventions. Consultants should work in the field of economics, animal health economics, epidemiology, surveillance and prevention strategies and sociology.

Regional Training Course on the Diagnosis of Avian Influenza (AI), Europe

Technical Officer: Gerrit Viljoen

The meeting will be held from 22 September to 2 October 2009 at Seibersdorf, Austria.

The two weeks theoretical and practical course, which will be organized by Joint FAO/IAEA Division with the financial support of the EC, aims at enhancing knowledge on highly pathogenic avian influenza (advanced molecular diagnosis of the virus sub-types by use of nuclear and nuclear related serological and molecular technologies), characterization and phylogenetic analysis and relationships of the virus sub-types, epidemiology of the disease. The ultimate goal is to contribute to the early detection and early reaction capabilities in Member States.

The course will be open to about twenty participants from the European Region (in particular CIS and Balkan countries) and countries participating in the ConFluTech project (Romania, Turkey, Armenia, Azerbaijan, Georgia, Islamic Republic of Iran, Iraq, Syrian Arab Republic, Jordan, Greece, Bulgaria) which are considered at risk regarding avian flu outbreaks. This advanced molecular diagnostic course is intended for participants with an academic background equivalent to a Bachelor's degree in veterinary, animal or biological science, and with experience in basic molecular biology techniques. Participants must be actively involved in animal disease diagnosis. The training course will be conducted in English, participants should be capable of freely expressing themselves and following lectures.

Regional (AFRA) Training Course on Transboundary Animal Disease Surveillance (RAF5057)

Technical Officer: Hermann Unger

The meeting will be held from 26 to 30 October 2009 in Dakar, Senegal.

Participants of the First Coordination Meeting of RAF 5057 (April 2009, Addis Ababa) concluded that disease surveillance is the best weapon to keep TAD's at bay. To perform proper surveillance and to evaluate and interpret data and results in a meaningful way, well trained epidemiologists are of major importance. Despite the training efforts in the 1990ies during PARC, the number of trained and qualified epidemiologists in the national veterinary institutions is dwindling. Prof. Koos Coetzer from University of Pretoria, Faculty of Veterinary Sciences, Dept. of Veterinary Tropical Diseases, agreed to help setting up such an epidemiology training course and it was understood, that there would be a need of 3 consecutive courses, namely "basic epidemiology and surveillance planning", "advanced epidemiology and reporting" and a final course on statistical methods and risk analysis. The course in Dakar will focus on disease specific surveillance strategies and the statistical background to create a valid surveillance plan.

In order to maximize the outcome, the same person should attend all courses to establish a core capacity in every laboratory. The course in Dakar will be open to all RAF 5057 participants and applications should be sent as soon as possible.

Consultants Meeting on Biology and Nutrient Requirements of Livestock During Compensatory Growth and Restricted Periods of Growth

Technical Officer: E. Nicholas Odongo

The meeting will be held from 12 to 14 October 2009 in Vienna, Austria. The objective of the CS is to develop a CRP to investigate the gut biology and nutrient requirements of livestock during compensatory growth and restricted periods of growth.

Regional Training Course on Clinical Pathological Molecular Technologies of Animal and Human Fasciolosis and the Treatment and Measures of Control (RLA5049)

Technical Officer: Gerrit Viljoen/Kathrin Schaten The regional training course will be held in La Paz, Bolivia, from 16 to 20 November 2009. The purpose of the training course is to harmonize and implement the best approaches and tools for molecular epidemiology, diagnosis, control and prevention of Fascioliasis, including theoretical and practical training and to determine the epidemiological spread of the disease, by classical and new generation techniques, and to identify areas at risk. Special attention will be given to clinical pathological molecular technologies and the treatment and measures of control.

The course will be open to participants from Argentina, Cuba, Mexico, Panama, Peru, Uruguay, Bolivia and other Latin American Countries (e.g. Ecuador, Honduras, Venezuela and Guatemala) which are considered endemic with fascioliasis.

This course is intended for participants with an academic background equivalent to a Bachelor's degree in veterinary, animal, human or biological science, and with experience in molecular biology techniques. Participants must be actively involved in the diagnosis and control of fascioliasis. Participating countries are encouraged to submit more than one application.

Past Events

Coordination and Planning Meeting (RER5015)

Technical Officer: Gerrit Viljoen

The meeting took place in Vienna, from 26 to 30 January 2009.

The Coordination and Planning Meeting reviewed the current situation on Avian Influenza (AI) in the Europe region, to outline existing nuclear techniques for early warning and surveillance, and to formulate a strategy and a detailed implementation plan for the new technical cooperation project RER5015 'Supporting Early Warning and Surveillance of Avian Influenza Infection in Wild and Domestic Birds and Assessing Genetic Markers for Bird Resistance' for 2009–2011. Sixteen designated counterparts (CP) from 14 countries attended the meeting.

The meeting covered general and technical discussions on the AI diagnosis and the tracing of migratory birds in the region; presentations and discussions of the Technical Cooperation (TC) Programme of IAEA including its components and procedures; country presentations covering the country AI situation, capacity of national institutes and suggestions for RER5015 project design. A scientific visit to the Seibersdorf laboratory of IAEA and a technical talk there were also held.

The following issues were discussed and agreed:

Objectives of the programme: should be development of the diagnostic and surveillance capacity of participating Member States. While the latter is dependent on the policy and strategy of each government, CPs can certainly contribute to the improvement of such capacity, by, for example, tracing the migrate birds by a stable isotope.

Indicator: At the end of the project cycle (end of 2011), the results of the programme, in light of the set objective and outcome, should be presented in a tangible form satisfying the international/regional standards, verified by reports submitted and complied by the Member States and evidenced by proficiency tests, accreditation or any other forms.

Regional Training Course on Molecular Epidemiology and Transmission of Fascioliasis (RLA5049)

Technical Officer: Gerrit Viljoen/Kathrin Schaten The regional training course was held in Montevideo, Uruguay, from 9 to 13 March 2009.

The purpose of the training course was to harmonize and implement the best approaches and tools for molecular epidemiology, diagnosis, control and prevention of Fascioliasis, including theoretical and practical training, to determine the epidemiological spread of the disease, by classical and new generation techniques and to identify areas at risk. Special attention was given to molecular epidemiology and transmission of Fascioliasis. Twenty-two participants from Argentina, Bolivia, Cuba, Honduras, Mexico, Panama, Peru, Uruguay, and Venezuela attended the training course.

Background and justification:

Fasciolosis is a parasitic disease affecting domestic ruminants worldwide. In South America it is caused by the liver fluke *Fasciola hepatica* and causes serious losses in cattle, buffalo, sheep and goats, posing a major threat to the livelihood of resource-poor farmers. The parasite lives part of its life in aquatic snails which act as intermediate hosts. Livestock are likely to acquire infection in and around wet areas, such as waterholes and streams where they gather to drink.

Liver flukes are a serious constraint to livestock production, with losses of \$600 million annually affecting both meat and dairy animals by mortality, ill thrift and the costs of veterinary intervention. There is a public health aspect to the problems caused by fasciolosis; it is also an important food borne zoonosis, classified by the World Health Organization as a re-emerging disease, with over 17 million people affected worldwide. The highest prevalence in humans is recorded in South America, in which two countries, Peru and Bolivia are most affected.

Attempts to control fasciolosis have not been entirely successful and the disease persists and is spreading in many areas. There is no commercial vaccine available and control relies on anthelminthic drugs and appropriate management where this possible. In Latin America, given its importance to both human and animal welfare, there is an urgent need for concerted, collaborative efforts to design strategies to diagnose and treat infections with a view to controlling the diseases caused by *Fasciola hepatica*. An opportunity to discuss various control options (both current and future) was provided by the training course organized under the Technical Cooperation Project on Integrated Control of Fasciolosis in Latin America (in Support of National Programmes) - RLA5049

Participants:

Mr Pablo Cuervo, Ms Erika Deis and Mr. Roberto Mera Y Sierra (Argentina), Ms. Maya Espinoza and Mr. Nicolás Lizon (Bolivia), Mr. Luis Mendez and Ms. Lazara Rojas (Cuba), Ms. Patricia Galvez (Honduras), Mr. Osvaldo Ibarra, Mr. Raul Rojas and Mr. Lino Zumaquero (Mexico), Ms. Katy Torres (Panama), Ms. Amanda Chavez, Ms. Lidia Conza and Mr. José Espinoza (Perú), and Ms. Luisa Gonzalez (Venezuela) were the sixteen participants from the 8 latin american countries which attended the training course.

The national participants were Mr. Daniel Acosta, Mr. Ulises Cuore, Mr. Jaime Sanchis, Ms. María Solari, and Ms. Soledad Valledor.

Results:

Molecular aspects of the epidemiology of fasciolosis were a major subject of discussion in the meeting. The search for molecular markers to define genetic diversity was regarded as a potentially useful tool that could be used to better understand the transmission of Fasciola hepatica in regard to its circulation between different definitive hosts (i.e. livestock), the intermediate snail hosts as well as possible reservoirs. A number of different markers for genetic characterization of Fasciola species were described.

A general review on the post-genomic era with examples of applications of gene technologies and the relationship between genomic and transcriptomic data was of great help when transferring these concepts to F. hepatica. The difficulties of studying parasitic helminth genomes was emphasized the potential value of the existing sequences in flatworm databases were shown.

The mechanisms by which double stranded RNA switches off an endogenous gene by eliminating the target mRNA was also explained. Studies from Uruguay suggested Fasciola cathepsins would be a good target to switch off. The usefulness of functional genomic tools such as iRNA when trying to control the disease was discussed.

For many years, the development of a vaccine to control of helminth parasites has been one of the most important challenges for immunologists and parasitologists. Preliminary results of a promising vaccine based on leucine amino peptidase antigen (LAP) developed in Uruguay showed that in sheep it was possible to obtain 87% protection.

The pharmacokinetics, pharmacodynamics and toxicity of the available anthelminthic drugs were explained and discussed. The differences in availability of these drugs in different countries was remarkable, with some countries in which few or no drugs were available (Cuba and Venezuela), and others where almost all of them were available, sometimes in combination with other drugs (Argentina and Uruguay). Participants were therefore able to provide discussion on different examples of the use of anthelminthics according to different management systems, including experience of the occurrence and detection of drug resistant parasites.

Results of an experiment carried out where susceptible and resistant adult F. hepatica were incubated in a buffer containing the anthelminthic Triclabendazole (TCBZ) were shown and the amount of drug metabolized by the resistant flukes was shown to be significantly less compared with susceptible parasites. The possible mechanisms were discussed. Glycoprotein P is thought to improve the metabolism or elimination of TCBZ and therefore may be responsible for the resistance. In another experiment, Triclabendazole was combined with Ivermectin. The resistant parasites had a higher concentration of Triclabendazole which suggests that Ivermectin was somehow facilitating the uptake of the drug, whereas there was no difference in susceptible parasites.

Integrated Pest Management strategies were introduced as a concept that field veterinarians will have to use in future. It would include surveillance of the parasitic status in the herd, the most effective form of treatment and its effectiveness, along with economic benefits. There should be a shift in the concept of control of fasciolosis, whereby the aim should be to obtain optimum profits in terms of productivity, instead of maximum profits, as this will more likely take into account many other factors such as sustainable production and environmental protection.

Concluding remarks:

Considering the importance of this parasite in Latin America, economically and to both animal and human health, this course was an opportunity to update the participants' knowledge on new technologies that appear to show promise in improving disease control through various vaccine technologies including irradiated metacercariae and silencing certain genes by the use of iRNA. Aspects on the epidemiology of Fasciola hepatica were also discussed. The correct use of the currently available drugs was seen as a priority in order to minimize the risk of creating a more resistant fluke population. Also, it was considered that an Integrated Pest Management approach should become the norm, when designing future control programmes.

Consultants Meeting on Stable Isotope Analysis (SIA) as a Means of Tracing the Migratory Movement of Waterfowl Involved in the Spread of Highly Pathogenic Avian Influenza (HPAI)

Technical Officer: A. Luckins

The meeting took place from 4 to 6 May 2009 at the VIC, Vienna.

Understanding the role that wildlife play in the ecology of AI viruses requires much more detailed knowledge of the movements of wild birds over their migratory flyways and it is important that we increase our studies to identify those routes, the stopover points and nonbreeding areas in several continents. This is a major challenge, and although considerable data have been accumulated by capture-recapture and ringing studies gathered over many years, other techniques, including radio telemetry, have been applied more recently in order to increase the accuracy of the information. Such methods are expensive however, and can be used on relatively few individuals. An alternative technology that can deliver information on bird origins and possible stopover points, and requires only simple sample collection, is stable isotope analysis (SIA). The purpose of meeting was to discuss the use of stable isotope analysis to determine migratory connectivity in wild water fowl and the implications for understanding the role of wild birds in the dissemination of highly pathogenic avian influenza (HPAI) and the ways in which it could be incorporated into existing TC projects on AI and into future CRPs. Six consultants attended the meeting Dr Nicolas Gaidet, Dr Jackie A. Clark, Dr Marius Gilbert, Dr Len Wassenaar, Dr Jong-Yun Kim and Dr Micha Horacek together with staff from the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, and Dr Scott Newman from FAO.

The consultants emphasised that this initiative involved a multidisciplinary approach, requiring expertise in ecology, ornithology, epidemiology, virology, geochemistry to enable understanding of the processes involved. The IAEA was recognized as being a key player in initiating and coordinating such a programme given its current involvement in training and research in the epidemiology of Avian Influenza. The IAEA could also fulfil another important role in stable isotope analysis by producing isotopic working standards for primary reference materials containing exchangeable hydrogen. The need for such materials is urgent and presents a considerable challenge if the use of δD isotope analysis in migration studies is to become a reliable tool for accurately predicting the origin of wild birds and providing a clearer idea of their epidemiological importance in HPAI. Arguably, achieving this aim could be a prerequisite to embarking upon longitudinal stable isotope studies involving different laboratories. Although a number of keratin laboratory standards have been prepared from cow hoof, chicken feathers and whale baleen as in-house working standards in a single laboratory, they are not available globally as primary reference materials for δD analysis and at present there are no internationally recognized organic standards for δD . Therefore it will be necessary to prepare several keratin standards in sufficient quantities to satisfy demand from isotope laboratories for periods (at least 10 years) beyond the immediate requirements of the SIA programme, with stable isotopic homogeneity, and a wide isotopic range for δD .

IAEA would also provide guidance in ensuring sample collection and processing of feathers followed recommended, standard procedures.

Stable isotope analysis provides an extra dimension to the data being collected on the migration of wild fowl using more conventional methods such as satellite tracking, transmitters and ringing and certainly, during the initial phase of a study, would provide complimentary information to that already assembled by those methods. Most importantly, SIA will allow investigation of feather specimens already collected and stored by AGAH EMPRES Wildlife Unit of FAO. The consultants considered that liaison with AGAH in developing the programme on SIA should be a priority consideration for APHS since, in addition to the archived specimen collection, the unit also has information on the resources, expertise and migratory bird flyways from different countries that could become participants in the SIA studies.

Primary consideration for participants in the SIA programme should be directed towards those countries in regions that are known "hotspots" of HPAI, i.e. locations where AI persists or frequently reemerges in Africa (Egypt and Nigeria) and East and South East Asia (Vietnam, Thailand, India, Bangladesh, China, Indonesia), and countries that are on migratory wild bird flyways including Mongolia, Kazakhstan and other countries in Central Asia and the Black Sea and Baltic Sea basins.

Although there have been a number of studies to identify wild bird populations infected with HPAI, it has not been possible to detect the virus in birds apparently healthy animals, but only in those that have been found dead, or *in extremis*. Nevertheless, the AGAH programme of marking and releasing wild birds with tracking devices has provided detailed analysis of the movements of wild birds throughout Eurasia and this information is a valuable guide in enabling selection of target species for isotope feather analysis. The number and range of bird species is quite large, although in some instances there is species overlap between different countries and regions.

The list comprises the following:

- India Garganey (*Anas querquedula*), Common Teal (*Anas crecca*), Eurasian Wigeon (*Anas penelope*), Gadwall (*Anas strepera*), Northern Shovellor (*Anas clypeata*), Northern Pintail (*Anas acuta*) and Ruddy Shelduck (*Tadorna ferruginea*)
- Egypt Common Shelduck, Eurasian Wigeon, Northern Shoveller, Common Teal, and Marbled Teal
- Nigeria Garganey (*Anas querquedula*), White-Faced Whistling Duck (*Dendrocygna viduata*) and Spur-Winged Geese (*Plectropterus gambensis*)
- China Northern Pintail, Eurasian Wigeon, Common Teal, Spot-Billed Duck, Mallard, Pintails, Ruddy Shelduck and Bar-Headed Geese
- Mongolia Bar-Headed Geese
- Kazakhstan Mallard (*Anas platyrhynchos*), Ruddy Shelduck (*Tadorna ferruginea*), and Gadwall (*Anas strepera*).

A key issue of the SIA analysis is how to assign birds of unknown origin to particular geographic sites i.e. their point of origin. In some cases this might be possible if there is isoscape data already available for known breeding and collection sites, but, provision should also be made for environmental sampling or ground truthing, using sites that the FAO programme can access. This will require samples of approximately 2 mL of water from deep wells in the region of the birds' habitats. These will be in up to 10 sites that are considered to be important in relation to known HPAI outbreaks. Environmental sampling should also be carried out where wild birds stopover and where domesticated poultry occur.

Specimen collection from wild birds should be limited in respect of tissue type; only feathers should be collected. Ideally, the collection will comprise 15 samples/species/site, but this will be affected by the situation on the ground at the time of collection. Consideration should also be given to sample collection from free-ranging domesticated poultry in locations where wild birds congregate as a stopover point in their migration.

Initially, the SIA survey should be limited to examining feather stable isotopes in flight feathers. Determining either $\delta 18O$ and δD ratios will provide information on migratory connectivity for wild birds, but since results for δD are easier to analyze, this isotope would be used for analysis (and taking into account needs for stan-

dardization). The need for the multidisciplinary approach is very apparent in interpreting the data accumulated in relation to feather sampling. It will be essential to understand the moulting pattern of captured birds in order to interpret SI data effectively. Juvenile birds and adults will need to be identified by competent ornithologists since the former can provide an indication of SI in breeding areas, whereas the adults can reveal data applicable to moulting sites.

Consultants Meeting on Irradiated Vaccines and their Potential for Use in the Control of Livestock Diseases

Technical Officer: Antony G. Luckins

The meeting took place in Vienna, from 11 to 13 May 2009.

The purpose of the meeting was to examine vaccine technology in relation to radiation attenuation in order to consider its potential as a technique that will stimulate the adoption of new initiatives to vaccination and control of animal diseases. This is particularly appropriate in relation to certain problematic diseases such as Rift Valley Fever and parasitic infections where vaccine technology is not very effective. Furthermore, attenuated vaccines are known to be potent immunogens and their use has led to the eradication of a number of global infectious diseases of animals and man. Three consultants, Dr Zhao-Rong Lun, Dr Alan Wilson and Dr Eric James attended the meeting together with staff from the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture and Dr Adama Diallo from FAO, Rome.

The consultants considered that IAEA was uniquely positioned to play a leading role in the implementation of a programme to achieve the above goals, and the initiative a timely one, firstly because in most instances recombinant vaccines have not yet lived up to their promise in delivering effective vaccines, and secondly, there is a considerable body of evidence in the literature to indicate that radiation-attenuated vaccines are effective and feasible. Moreover, there is a resurgence of interest in this field with the current development of the first human radiation-attenuated anti-parasite vaccine, for malaria. The consultants considered the economic impact and potential for vaccine development in a comprehensive range of viral, bacterial and parasitic pathogens of livestock and made recommendations for further development. A key issue in producing attenuated vaccines will be the requirement to develop improvements in radiation technology and to adopt standardized procedures for the preservation, including cryopreservation, handling and transport of immunogenic material. These procedures, together with the need to develop biological measures of vaccine potency are likely to be an important part of the programme of activities carried out at the Seibersdorf Laboratories.

Diseases caused by pathogenic animal trypanosomes continue to be a major constraint on livestock produc-

tivity in Africa, Asia and South America. Antigenic variation of the African trypanosomes presents a serious obstacle to vaccine development but the diseases caused by non tsetse-transmitted trypanosomoses T. evansi and T. vivax merit attention. This objective circumvents the need for improved technologies to culture metacyclic trypanosomes as it will rely entirely on bloodstream forms of the parasites. This will require an experimental approach to examine parasite attenuation involving optimization of irradiation 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. This process will be facilitated by development of labelled (radioactive or intrinsic fluorescent) trypanosomes for tracking studies. Humoral and cell-mediated immune responses will need to be characterized by conventional techniques. Vaccinated animals will need to be given a realistic challenge to evaluate the level of protection. Assuming protective immunity can be achieved then further development will require improvement of existing cryopreservation protocols for the storage, transport and distribution of the irradiated trypanosome vaccine. In the case of Theileria various procedures, including infection and treatment regimens, are used to induce immunity in livestock utilising sporozoites or lymphocyte stages of the parasites. A radiation attenuated Theileria annulata vaccine has been experimentally tested, but there is a need to evaluate the literature in respect of the currently available techniques and the potential for a new approach involving radiation-attenuation.

The most successful example of an irradiated vaccine is that developed against infection with the lungworm, Dictyocaulus vivaparus, that has been used successfully for some 50 years. Amongst the many helminth parasites that could be considered as potential candidates for developing an irradiated vaccine the consultants focused primarily on Fasciola spp because they considered a vaccine was technically feasible, and the disease important due to its worldwide economic impact and in some regions it is also a zoonosis. Two other helminth parasites, Schistosoma bovis and S. japonicum, present additional targets for attenuated vaccines for livestock. The life cycles for all of these parasites are similar, involving snail intermediate hosts that produce the infective stages, namely metacercariae for Fasciola and cercariae for Schistosoma and these stages are the target immunogens for vaccine development.

Preliminary studies in Sudan with low numbers of radiation-attenuated metacercariae of *F. gigantica* have already shown promise in protecting cattle (SUD5028). Based on these studies it should be technically feasible, with minor modifications of existing technology, to scale-up the work to include expanded field studies. Before this can be accomplished experimental studies are required specifically to a) determine the optimum attenuating dose of irradiation and, b) the number of parasites to use for oral vaccination - defining these

parameters is essential prerequisite to this work. Monitoring the immune response and metabolic parameters including levels of serum transaminases resulting from parasite-induced pathology, as well as numbers of eggs shed would provide measures of vaccine efficacy. Preliminary studies to characterize the optimum irradiation attenuation dose and procedure will need to be conducted initially in Seibersdorf working with *F. hepatica*. Experimental studies on vaccination could be conducted in rabbits.

Of all the bovine schistosome parasites, *S. bovis* is considered the most important in terms of morbidity and mortality. Studies conducted with *S. bovis* in Sudan in the 1970 - 80s demonstrated significant protection using radiation-attenuated schistosomula in both laboratory and field studies. These studies demonstrated the economic cost effectiveness of vaccination against

S. bovis in Sudan. 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 studies have also been conducted on *S. japonicum*. These results were promising, however, there are considerable problems with the snail intermediate host: small size of snail, difficult to grow, small numbers of cercariae/snail, stickiness of cercariae, all of which lead to very poor yields of parasites. *S. japonicum* is however an important parasite as a zoonosis and a parasite of water buffalo that acts as a reservoir of human infection in China.

There is also scope for developing a radiation attenuated vaccine against *Haemonchus*, a common parasite and one the most pathogenic nematodes of ruminants. However, recent developments in studies on cryptic gut antigens have shown promise and might preclude the need to develop an RA vaccine.

With regard to viruses, there is a generic need to investigate the use of irradiation to kill viruses as an alternative to e.g. formalin because of the strong possibility it will leave the viral proteins in their native conformation. This would be anticipated to greatly enhance their immunogenicity and efficacy. The reduction in titre due to inactivation would be less for irradiation than for chemical treatment. This would impact on the cost of manufacture and result in dose-sparing in relation to virus production.

To increase the efficiency of the irradiation process there is a need to develop flow-through methods of irradiation rather than batch processing for viral killing. It would also enable easier monitoring of the killing process by means of *in vitro* culture. For some viral vaccines where there is poor protection and the safety margin is small, irradiation can lead to safer and more efficient products.

Priority should be given rift valley fever, an important zoonotic disease, and foot and mouth disease which are major pathogens of livestock in Africa and the whole world respectively. Vaccines exist for both of these diseases but significant improvements are needed. Irradiation could speed up production process that would be useful in dealing with emergency situations and provide better standardisation and immunogenicity of vaccine strains.

Radiation killed bacteria are more effective immunogens than chemically treated organisms as has also been shown with viruses, indicating the potential advantages of radiation attenuated vaccines. UV irradiation has proven an effective attenuating modality for *Leptospira*, but indications are that gamma irradiation would provide much better conditions for attenuation and it is recommended that this should be developed further.

Research Coordination Meeting on Development and Use of Rumen Molecular Techniques for Predicting and Enhancing Livestock Productivity 2003–2009 (D3.10.24)

Technical Officer: E. Nicholas Odongo

A large proportion of the global ruminant populations are located in tropical environments, where animals feed predominantly on low quality highly fibrous forages. However, studies have shown that methane emissions from ruminants fed on highly fibrous diets are higher than those feed better quality diets. Furthermore, the production of methane in the rumen can represent a loss of up to 15% of the digestible energy depending on the type of diet. Therefore, reducing methane production could benefit the ruminant energetically provided the efficiency of ruminal metabolism is not compromised. The objective of this CRP was to reduce methane (a greenhouse gas) emission from livestock and divert the energy being lost in methane production towards increasing livestock production thus enhancing the efficiency of production and reducing environment pollutants.



1 Back row, left to right: H. Makkar, K. Joblin, D. Kamra, J. Liu, R. Long, P. Kongmun, R. Mackie, M. Mitsumori, M. Kreuzer. Front row, left to right: N. Odongo, G. Ayelet, D. Delgado and O. Mayorga. Not in the picture are A. Abdalla who took the photograph and C. Mcsweeney who had to leave to catch his flight.

A planning meeting and training workshop were held from 19 to 30 April 2004 at the CSIRO's Livestock Industries in Brisbane, Australia. First three days (19 to 21 April) were devoted to the planning meeting whose objectives were to: a) acquaint the Research Contract and Agreement Holders with on-going work and the resources available to the participants in terms of staff, laboratory equipment and funding, and b) plan future studies.

This was followed by a training workshop from 22 to 30 April 2004 which was attended by nine Research Contract Holders (RCH). The workshop consisted of lectures and practical exercises on:

- conventional anaerobic microbiology techniques
- culturing techniques for methanogens
- PCR based molecular ecology techniques
- quantification of rumen populations by real time PCR, and
- measurement of methane production from animals and cultures

The first Research Coordination Meeting (RCM) was held from 12 to 16 September 2005. The purpose of the RCM was to review the work done and plan future work. This meeting was attended by eight RCH, four Research Agreement Holders and one Consultant. During this meeting, detailed work plans for the next phase (until November 2006) for each RCH were developed. A second training workshop to build RCH's capacity to measure methane production in vivo was held from 26 September to 7 October 2005 at the Institute of Animal Science and Nutrition, ETH-Zentrum, Zurich, Switzerland. The workshop consisted of lectures and practical exercises on:

- SF6 tracer technique
- respiration chambers
- tunnel System for methane determination using an infra-red detector and GLC
- chamber/box system for methane determination using a GLC
- indirect method for methane determination by infusion of labelled short chain fatty acids
- direct method for methane emission by infusing labeled methane

The final RCM was held at the Vienna International Centre in conjunction with the International symposium on Sustainable Improvement of Animal Production and Health from 10 to 13 June 2009. It was attended by all eight RCHs, five of the six Research Agreement holders and one Technical Contract holder who presented and discussed work progress and the final reports. Mr. E. Nicholas Odongo was the Scientific Secretary of the meeting.

Achievements:

- 1. In vitro gas production test have standardized and used for screening of plants containing secondary metabolites by all RCH.
- 2. Real-time PCR to enumerate rumen microbes in in vitro and in vivo systems, and DGGE for studying rumen microbiota diversity were optimized.

- 3. Protozoal activity by (14C) Radio Isotope Technique have been standardized and were used for screening.
- 4. In vivo methane production measurements in small and large ruminants using SF6, tunnel system and open circuit respiration calorimeter were validated and used.

Outputs:

- 1. Over 30 students were trained to MSc and PhD levels during the CRP.
- 2. Two book/manuals were published and over 50 papers published in peer reviewed journals, and
- 3. Over 50 project staff, technicians and researchers were trained in molecular biology techniques.

General conclusions:

- 1. The protocols for enumeration of microbial population, study of microbial diversity and in vivo measurement of methane using nuclear and nuclear related techniques were collated in the form of a manual during this CRP. Training workshops to build participants capacity proved to be very useful and led to the uniforma, accurate and successful use of these methodologies in all the laboratories.
- 2. Methodologies developed in the CRP (e.g. in vitro screens, molecular ecology tools) have been refined for future nutritional evaluations of feedstuffs. These techniques also have direct application in the evaluation and implementation of anaerobic bioreactors for methane producing systems. However, studies that quantitatively and mechanistically link phylogeny and function of H_2 production and utilization are urgently needed. More research in functional genomic approaches is warranted to improve our understanding of the concepts and mechanisms involved in interspecies H_2 transfer. We need to know: who is there, who is active and what are they doing.
- 3. Three low cost in vivo methane measurement methods i.e. SF6, tunnel system and open circuit respiration calorimeter have been constructed and validated for accuracy in the CRP. These systems could be fabricated locally and used in Member States.
- 4. In vitro methods for screening of plants and plant products for antimethanogenic activity have been standardised. These are low cost methodologies and have potential for use in laboratories equipped with basic facilities.
- 5. More than 200 plant and plant extracts comprising of browse, multi purpose trees, medicinal plants and spices from Asia, Africa and South America were screened for the effects of plant secondary metabolites, specifically (1) polyphenol-containing plants, (2) simple phenols in the form of phenolic acids, (3) purified tannins, (4) saponin-containing plants, and (5) isolated saponin-rich fractions as well as of bromocloromethane on methanogenesis. Methane production in vitro was reduced by between 10 and 100% whereas in vivo, methane pro-

duction was reduced by 11 to 35%. The results indicated that *Terminalia belerica*, *Terminalia chebula*, *Emblica officinalis*, *E. Jambolana*, *Quercus incana*, *Populus deltoides*, *Foeniculum vulgare*, *Syzygium aromaticum*, *Allium sativum*, *Psidium guajava*, *Canabis indica*, *Trachyspermum ammi*, *Mangifera indica*, *Mentha pipperita* and *Eucalyptus* globulus inhibited methane production by 25 to 100% and a large number of plants were also inhibitory for ciliate protozoa. These have potential to decrease methane production in ruminants. However, more long-term studies using these strategies are warranted including on-farm evaluation, cost-benefit analysis and product quality.

Research Coordination Meeting on Gene-based Technologies in Livestock Breeding: Characterization of Small Ruminant Genetic Resources in Asia 2005–2009 (D3.10.25)

Technical Officer: Mario Garcia

The final RCM was held at the Vienna International Centre, from 8 to 13 June 2009, in conjunction with the International Symposium on Sustainable Improvement of Animal Production and Health. Research, Agreement and Technical Contract holders presented and discussed the final reports.

Small ruminants such as sheep and goats are a critical livestock resource in Asia as they substantially contribute to the livelihoods of millions of small farmers on the continent. There is a notorious genetic biodiversity in small ruminants in developing countries, much of which controls advantageous traits influencing adaptability to harsh environments, productivity or disease resistance; however, many of these populations are usually neglected and underutilized in crossbreeding with specialized breeds. Most of the indigenous goat and sheep breeds have been poorly evaluated and therefore, little is known about their genetic potential on important productive traits.

More knowledge about the genetic and phenotypic characteristics of the breeds of sheep and goats must be available to ensure their optimal management and utilization. Characterization is one of the four Strategic Priority Areas in the adopted Global Plan of Action for Animal Genetic Resources of FAO, indicating the critical importance of this activity. On the other hand, characterization activities can only be used optimally when they are included as part of a multi-national effort. In this respect, the main objectives of this CRP were:

- To get the phenotypic characterization of certain sheep and goat breeds of Asia, which will complement existing FAO and ILRI databases for Africa and Europe;
- To develop capacity within the Asian region to use radio-isotopic micro-satellite methods and related

technologies for genotype characterization of ruminants;

- To complete the analysis of regional and global genetic diversity of each species based on molecular data;
- To assess new technologies for diversity assay; and make recommendations on their future application for improving ruminant productivity.

Activities under the CRP included an initial field survey to identify and enlist local breeds, finding out the distribution pattern within the country, collect morphological and productive data of individuals and populations, including digital photographs of all animals, and collection of blood samples for DNA extraction and quantification. Also, laboratory work included DNA microsatellite, mitochondrial DNA and SNP analysis, and then, statistical analysis for estimating genetic distance and diversity, traits uniqueness, and genotype x phenotype x environment correlations.

The CRP was integrated by eight Research Contract holders representing Bangladesh (Mr. Omar Faruque), China (Shu-Hong Zhao and Yue-Hui Ma), Indonesia (Mr. Muladno), Islamic Republic of Iran (Mr. Seyed Ziaeddin Mirhoseinie), Pakistan (Ms. Arif-un-Nisa Mavqi), Sri Lanka (Ms. Pradeepa Silva) and Vietnam (Ms. Le Thi Thuy); two Research Agreement holders (Mr. Paolo Ajmone Marsan, Italy, and C. Devendra, Malaysia); and five Technical Contract holders (Mr. James Kijas, Australia; Mr. Fernando Garcia, Brazil; Mr. Mehdi Fazeli Niaki, Islamic Republic of Iran; Mr. Sigbjorn Lien, Norway; and Mr. Olivier Hanotte – Mr. Han Jianlin, ILRI-Nairobi). Mr. Mohammed Al-Shaik (Saudi Arabia) has participated as an observer.

A training course was held at the start of the project at the International Livestock Research Institute (ILRI) in Nairobi, Kenya to provide theoretical training in molecular methods for assessing genetic diversity, and hands-on on animal sampling, DNA preparation and storage, biodiversity analysis, and identification of genes by QTL mapping. A second training course was held in Beijing (ILRI), China for complementing activities and for genotyping part of their samples. In total, 17 research contract holders and associated scientists benefited from these two training courses and 42 laboratory staff in eight Asian countries have the capabilities to use radio-isotopic micro-satellite methods and related technologies for genotype characterization of ruminants.

The meeting was attended by all research contract holders with the exception of the Iranian representative, the two agreement holders, and two technical contract holders (Mr. Fernando Garcia and Mr. Han Jianlin). The meeting was also attended by Mr. Massoud Malek (Animal Production Unit, IAEA Seibersdorf Laboratories) and Mr. Paul Boettcher (FAO). Mr. Brian Sayre (Virginia State University, USA) participated as observer and Mr. Cao Jianhua on behalf of Ms. Zhao. Mr. Mario Garcia Podesta was the Scientific Secretary of the meeting. Several tools were developed through the course of the programme. For instance, a single nucleotide polymorphism (SNP), and micro-satellite and mitochondrial DNA analysis including standard operating protocols (SOPs) were developed at the Universidade Estadual Paulista (UNESP-Brazil), and a Real Time database for Quantitative Trait Loci/Genes/DNA sequences and genetic characterization in small ruminants was developed by Kasra Web (Islamic Republic of Iran). Also, the participation and contribution of ILRI, both in Nairobi and Beijing played a key role as research contract holders were able to genotype themselves part of their samples. At this stage of the CRP, all laboratory participants have minimal functional equipment for DNA extraction, quantification PCR, and electrophoresis.

In total, 4500 samples from 40 sheep breeds and 60 goat breeds from Bangladesh, China, Indonesia, Islamic Republic of Iran, Pakistan, Saudi Arabia, Sri Lanka and Vietnam have been genotyped at ILRI-Nairobi and CAAS-ILRI-Beijing using 15 microsatellite DNA markers. Some of the results have shown the highest genetic diversity in two Iranian populations; medium genetic diversity in six Chinese, five Bangladeshi, four of the five Pakistani and four Sri Lankan indigenous populations; and the least genetic diversity were found in five Vietnamese and four Indonesian populations.

Gene differentiation (Fst estimator) among goat populations was high (9.8%) and genetic differentiation (pairwise Fst estimates) was highly significant (p<0.01) for all pairs of populations between countries. Within country, the genetic differentiation was also significant (p<0.01) between all populations except between the two Iranian and the four Bangladeshi populations. Admixture analysis clustered the Asian goats into three distinct genetic groups: the first included all populations from Iran and China; the second the five Vietnamese and three Indonesian populations; while the remaining Indonesian and all Bangladeshi, Pakistani and Sri Lankan populations belonged to the third group. The results suggest that the domestic goats may have followed several separate ancient migration routes of dispersal into the Asian continent.

Despite this RCM was the final official meeting, there are some additional work to be concluded before proper final reports can be submitted for publication. The laboratory work related to the microsatellite analysis is completed but part of the data is still being organized by Mr. Han Jianlin. The mtDNA sequencing analysis, done in Brazil, will be ready by July-August when the remaining PCR or DNA samples from four countries are shipped in dried plates to Fernando Garcia's laboratory. Also, the phenotypic database is being organized by Mr. Omar Faruque and will be ready next month after receiving missing data from some populations.

In order to compile all the results and documents for preparing the final country reports, Messrs. Han Jianlin and Yue-Hui Ma kindly offered to sponsor a 2-3 week workshop in Beijing (under Chinese government sponsoring of air tickets and maintenance costs) in October 2009. The IAEA and CRP participants are greatly thankful to this kind initiative which will allow finalizing research data on sheep and goat breeds characterization produced by eight Asian countries in the last five years. The group has decided to publish the country reports as scientific papers in a special edition of a peer reviewed journal (possibly Small Ruminant Research – SRR, or alternatively Genetics, Selection and Evolution – GSE), plus a general article on breeds from Asia and the respective production systems, in place of the IAEA-TECDOC. It is also planned to produce two scientific articles about sheep and goat population genetics.

Research Coordination Meeting on Veterinary Surveillance of Rift Valley Fever 2005–2009 (D3.20.23)

Technical Officer: Gerrit Viljoen

The final RCM took place at the Vienna International Centre, from 8 to 13 June 2009, in conjunction with the International Symposium on Sustainable Improvement of Animal Production and Health. Research, Agreement and Technical Contract holders presented and discussed the final reports.



The classical methods for the detection of antibodies to RVFV include haemagglutination-inhibition, complement fixation, indirect immuno-fluorescence and virus neutralization tests. Disadvantages of these techniques include health risks to laboratory personnel due to exposure to infectious virus, as well as restrictions on their use outside RVF endemic areas. Although regarded as a gold standard, the virus neutralization test is laborious, expensive and requires 5-7 days for completion. It can be performed only when standardized stocks of live virus and tissue cultures are available. Consequently, it is rarely used and in only highly specialized reference laboratories. Delay in diagnosis associated with traditional virus isolation and identification techniques may represent a significant problem for regulatory healthcare authorities faced with an epidemic of RVF, especially outside its traditionally known geographic confines. Hence, considerable efforts have been made recently to develop techniques for the rapid and early detection and identification of RVFV. Serological and molecular diagnostic tests have the potential to resolve these problems.

In this CRP it was intended to focus on improving the diagnostic capacities of Member States. This was to be achieved by evaluating, validating and standardising existing and newly developed serological tests for the surveillance of RVF and by introducing and transferring suitable molecular as well as isotopic technologies for virus detection. Genomic and genetic techniques allow for rapid and early virus detection and genetic typing, without the risks of accidental laboratory infection. This should enable participants of the CRP to use harmonised protocols and procedures, and to exchange their research data and findings. The implementation of validated serological techniques (for example ELISA) was seen to help determining the RVF sero-conversion status of individual animals and herds, while the molecular techniques were to assist in the rapid and early detection of virus to enable the timely implementation of quarantine and control measures, including the differentiation between vaccine and field strains. The recent RVF outbreaks in Kenya (2006/7), Sudan (2007/8) and South Africa (2008/9) and the timely response by the diagnostic laboratory to inform and support the national authorities to effectively control the disease (human deaths were restricted to less than 150 in Kenya) underlined the importance of the CRP.

Focus in this CRP is on the evaluation, validation and implementation of RT-PCR and PCR sequencing procedures for early and sensitive detection of the RVF virus and its use in molecular epidemiology using isotopic techniques to improve diagnostic sensitivity (via isotope incorporation into PCR amplicons) and to confirm diagnostic specificity (via hybridization of amplicons with isotope labeled probes). In laboratories equipped with real-time PCR capabilities, the manual PCR procedures were adapted to include their use as part of the standard operating procedures (SOPs). Manual isotope based slab PCR-sequencing procedures were implemented (In laboratories equipped with automated sequencing equipment, these procedures were adapted for use). The specific objectives were: (1) Evaluation, validation and use of iELISA formats to detect virus-specific antibodies; (2) Evaluation of recombinant antigens for use in indirect and competition ELISA's; (3) Harmonization of Standard Operating Procedures (SOPs) and introduction of quality assurance procedures for RVF-ELISA and RVFV RT-PCR; (4) Setting up of a serological and molecular epidemiological database (based on antibody prevalence and virus isolate genetic variation).

Progress towards achieving the objectives is satisfactory: the IgG and IgM ELISA platforms were implemented, evaluated and validated; serological procedures were harmonized and are available including SOPs; RCH's were trained in molecular technologies and quality assurance management and are in the process of training the laboratory technicians (guidelines and a manual are available); the recombinant RVF antigen was evaluated and are under validation as substitute antigen for the ELISA platforms; epidemiological and surveillance (including sampling frame) strategies were developed and are implemented. More than 10.000 samples from RVF infected areas, or areas at risk, were analyzed using the CRP's serological and molecular assays and it was concluded that RVF is endemic in all participating MS and infections are constantly moving from one area to another - probably due to rainfall patterns and vector presence and due to livestock movements. Several national surveys are under way applying the diagnostic assays that resulted from this CRP's development work.

The final year's work plan will focus on the finalization of standard operating procedures (sample collection, testing, procedures, protocols, data analysis, etc), and to utilize these procedures as routine actions in the diagnostic laboratory for the early and quality assured diagnosis of RVF; the analytical and diagnostic validation of the recombinant nucleocapsid protein and virus like particles (VLP) in ELISA formats towards the epidemiological presence of the virus induced antibodies and disease to reduce the risk to livestock and people; implementation of protocols to perform RT-PCR/real-time PCR, isothermic PCR (idLAMP) and PCR-sequencing and safe handling of isotopes; implementation and maintenance of a surveillance protocol designed to facilitate early detection of RVF and its contribution towards the national disease control programme; continued support towards the FAO database (situated in Senegal), including the input of data from the CRP holders; the sending of RVF sera to Seibersdorf to serve as world reference sera; to evaluate the efficacy of an irradiated RVF vaccine and VLP's as a livestock vaccine. In addition, to the irradiated vaccine, the evaluation and use of DIVA diagnostics (differential infection and vaccine analysis) for 2 vaccines under test (esp. MP12 in the USA and Clone 13 in Africa) will be finalized.

International Symposium on Sustainable Improvement of Animal Production and Health

Technical Officers: Gerrit Viljoen/Kathrin Schaten The International Symposium was held from 8 to 11 June 2009 in Vienna, Austria.

It was attended by more than 400 participants from about 100 Member States of the IAEA and FAO, including several international organizations including WHO, OIE and ILRI, with oral and poster contributions. The main topics discussed were related to interactions among nutrition, reproduction and genotype, effects of environment on animal productivity, detection and control of transboundary, emerging and zoonotic diseases, and food safety and security in the 21 century. Twenty-four keynote speakers, 53 oral and 163 posters were presented and discussed highlighting the current global food security situation, animal production and health constraints, research needs and options



The Opening Session, 8 June 2009.

on how to reduce hunger and alleviated poverty in the world.

More that 2.5 billion people depend for their income and nutrition on the efforts of smallholder farming households... particularly on the work of women farmers. The United Nations, as indicated by the Secretary General Ban Ki-Moon, has considered food security as one of the major concerns and is supporting national and regional responses to food insecurity. As a result of this, emphasis has been placed on smallholder farmers because they produce the majority (up to 90%) of the food that in consumed in the world. It is important to note that food insecurity and hunger are being experienced by at least one billion people i.e. one in six people; with a child dying of malnutrition every six seconds.

Furthermore, meeting the ever increasing demand for animal products can only be achieved through the protection of animals from diseases, the selection of animals that give more meat and milk, and the optimal utilization of local resources whilst protecting the environment to which the Animal Production and Health Subprogramme contributes through the use nuclear and nuclear related techniques. The meeting was told the only ways forward is through integrated farming systems that match the farming system with available resources through multidisciplinary and interinstitutional collaboration. The symposium proved to be an important forum for the scientific community to report not just valid and key technical results but coherent research and applied research work conducted by the IAEA and FAO through links and partnerships with other UN organizations as WHO and OIE as well as with other international agricultural and research centres, in cooperation with Members States. Furthermore, better communication (and resources) on application of nuclear techniques on agricultural practices to discreet audiences is at the heart of wide-scale adoption of research products – DFID allocates 30% of its research budget to communications.

Papers and talks presented during the symposium showed that the on-going world food crisis is exacerbated by three important factors: the end of inexpensive energy era (and beginning of expensive inputs), global

climate change, and global resources depletion including mineral fertilizers, irrigation water, soil fertility, and land use. Our challenge is not only how to ensure adequate food for the current 963 million hungry people, but also how we are going to feed, a world population of over 8.3 billion people by 2030. If food demand is to be met in future, increased outputs will have to come mainly from intensified and more efficient use of the land, water and plant and animal genetic potential, fisheries and forestry resources that smallholder farmers in developing and transition countries have at their disposal. The IAEA contribution with the development and application of nuclear and nuclear related techniques are highly relevant and timely. However, we must differentiate the target audience for application of nuclear techniques as the users in many cases are different from beneficiaries. Research needs to be adapted to the local conditions taking into consideration cultural norms and differences on a region by region basis. Technologies and methodologies developed in the North cannot always be transferred to the South, but also, developing countries need to implement their own research on problems that do not exist in the developed world.

Facts, data, results, and recommendations presented both orally and on posters, and complemented with informative discussions and experiences from the plenary resulted in a long list of valuable ideas, proposals and procedures that can be used at various levels to boost livestock production, reduce hunger and improve health status in animals and humans. For example, dietary manipulation and nutritional strategies to reduce methane production while improving body weight gain and milk yields, management practices to improve fertility and number of newborns, using low inputs, and therefore benefiting farmers with higher economical returns and the world with larger quantities of safe and wholesome foods.

Meat and dairy products are mainly produced by cattle, pigs, sheep, goat, and poultry, but there are several other species which are region or country specific, which need to be properly characterized and their efficiency of production improved for the benefit of the local population. Among them, the buffalo is an important ruminant species in Asia that requires further research in both the effects of environmental conditions on milk production and reproductive efficiency or improved fertility using artificial insemination. Camels in the Arab world and camelids (alpaca, llama, and vicuna) in the Andean countries substantially contribute to meat, milk (camel) and fibre; yaks in China are essential animals for a vast number of people; and rodent species such as the cane rat in West and Central Africa and the Guinea pig in Peru and Bolivia are all important sources of meat.

Much of research data, methodologies, management practices, and health procedures produced and validated in the developed world cannot be easily transferred to developing countries, due to climatic, soil, genetic and cultural differences and therefore, the huge amount of valuable research has to be properly evaluated and in many situations adapted; however, in many other situations developing countries have to develop their own technology based on the existing available resources - food and feeds, species and breeds, and environmental conditions. As an example, embryo transfer technology, widely available in the North, is not economically feasible under smallholder farmer conditions despite efforts from Governments, NGOs and other interested parties. On this respect, the search for and evaluation of locally available resources as feedstuff for livestock, nutritional evaluation of feed resources, reduced dependence on expensive concentrates, use of alternative feed resources and agro industrial by-products, strategies to reduce methane production in ruminants and improve the efficiency of production, alternatives for oestrous synchronization, the use of artificial insemination on a fixed time without the need for heat observation, and the expansion and support of artificial insemination services were presented and discussed.

The second part of the symposium covered Animal Health, One Health and Food Safety. Nearly 40 oral and over 50 posters presentations covered a wide range of topics detailing work carried out in MS using stateof-the-art nuclear, nuclear associated and nuclear related technologies to address the various problems they face and also highlighting the work of international agencies.

The need to intensify and increase food production to feed the ever growing human population with reliable and save food is also going to demand more efficient means to diagnosis and control animal diseases and to measure food and feed contaminants. The most prominent challenges that arose from the symposium focussed on the increase in human population, new farming systems, increased movement of animals in world trade and the alterations in ecosystems brought about by climate change and the geographical distribution of pathogens or their vectors. In this scenario, resource poor developing countries will become increasingly vulnerable to emergencies from disease threats.

We are going to be faced with an increasing prevalence of infectious diseases due to both known, as well as hitherto unknown emerging diseases. A significant proportion of the latter, over 60%, are likely to be zoonotic diseases, the greatest proportion of which will probably be associated with wildlife and therefore the domestic and wildlife interface is very important (as examples: HIV, avian influenza, rabies, ebola, Rift Valley fever and others). This will challenge our perception of surveillance, as it will be imperative to be able to diagnose infections in the animals, rather than identify a problem zoonosis after it has spread to humans.

Faced with such problems, it will be important to prioritize in terms of resource allocation and focus on:

• The most important endemic diseases

- The most important zoonotic diseases
- The most important exotic diseases

The European Technology Platform for Global Animal Health (ETPGAH) is presently addressing this issue, with a strong European bias towards their relevance, and implementing information dissemination through national animal health meetings (e.g. in the UK the Association of Veterinary Teachers and Research Workers). Links with FAO, WHO and OIE will be essential to achieve some level of harmonization and success.

FAO is instrumental in bettering the lives of rural populations by improving agricultural productivity, and thereby contributing to the growth of the world economy, while OIE, through a network of Reference Laboratories and Collaborating Centres provides the scientific and technical information relevant to notifiable diseases important to trade. These organizations, with a mandate to improve animal health in the widest sense, also participate in an initiative with WHO in providing a Global Early Warning System for Major Animal Diseases, including Zoonoses (GLEWS) that links human and animal health systems.

However, there are still gaps in our knowledge, for instance in the global economic burden of zoonoses. Better insights are needed into the transmission pathways for opportunistic zoonotic infections, and at the same time it is essential to understand disease epidemiology in each host species, particularly wildlife reservoirs, since persistence of infection will slow down attempts to effectively control diseases. It was suggested that zoonotic diseases were under-reported and that many MS did not have the capacity to recognize and diagnose them, confirming the view that in this area more than any other, a cross-disciplinary approach was essential to fill knowledge gaps in research and diagnostic needs and define responsibilities between veterinary and public health authorities thereby ensuring integrated surveillance and control of diseases. Amongst the so-called neglected parasitoses are various helminth infections including Fasciola and Taenia, leishmaniasis and sleeping sickness that have a high impact globally or locally.

FAO's EMPRES programme for the progressive control of Transboundary Animal Diseases (TADs) involves the transfer of appropriate technologies and the training of veterinary technologists, sharing information of major animal disease threats, providing epidemiological analysis and assessments of major animal disease threats and providing access to prediction and prevention studies of major animal disease threats, including zoonoses. These include rift valley fever (RVF), peste des petits ruminants (PPR) and foot and mouth disease (FMD). For FMD regional road maps and Progressive Control Pathways (PCP) are being developed and will be in place in all seven major endemic regions by 2010. By 2020 it is expected that FMD will be under control and circulation of the virus reduced in many of the countries in these regions.

thereby allowing them to participate in safer and increased trade.

There is a comprehensive range of new generation (nuclear and nuclear associated and nuclear related serological and molecular) diagnostic techniques to detect animal diseases. The list is long and includes both direct and indirect methods. Considerable strides have been made at, for example, the University of Uppsala together with the Animal Production Unit of the IAEA's Seibersdorf laboratories and other partners, in developing some of these assays including PCR and ELISA tests for Avian Influenza (AI), FMD and food borne infections with Hepatitis E and others. On site diagnostics for FMD and AI using disposable automated sample preparation units have been developed with communications systems that enable instant reporting of results from even remote locations. Moreover, handling is optimized and indeed simplified to reduce human (field and health worker) and laboratory technician exposure. Rapid, specific pen-side diagnosis has also been made possible for PPR by the development of a loop-mediated isothermal amplification (LAMP) PCR.

A number of wild ruminant species are susceptible to RVF infection but it is not certain if these animals have a role in virus maintenance (i.e. as host) or transmission (i.e. as amplifying vehicle) during inter epizootic periods. Using a recombinant nucleocapsid based indirect ELISA it was possible to detect specific IgG antibodies to RVF virus in wildlife sera and, compared to the virus neutralization test, it had a very high diagnostic performance in various wildlife animal species. It therefore provides a useful tool for epidemiological studies of RVF virus infections in domestic and wildlife species. Such investigations might help to elucidate their specific role in the epidemiology of the disease including the mechanisms of the virus maintenance within the host-vector natural cycle.

An important development in our understanding of the epidemiology of capripox viruses (CaPVs) was provided by a gene-based classification to enable unequivocal identification of the origin of viral isolates. Using a real time PCR based on the glycoprotiencoupled chemokine receptor (GPCR) gene, it was possible to simultaneously detect, genotype and quantify CaPVs and classify them as sheep pox, goat pox or lumpy-skin disease viruses.

Global trade in livestock and livestock products will continue to increase as it is demand driven towards an ever increased protein based consumption. However, biosecurity and in particular, infectious disease, along with other factors will limit this trade. Since the greatest biosecurity risk is posed by the live animal, investment in processing capacity at or near livestock production sites and prior to export can significantly reduce the biosecurity risks. Trade in the processed product and not the animal could be a way for livestock producers to trade their way out of the poverty trap in developing countries. This would be of greatest benefit in those areas currently excluded from trading due to biosecurity concerns and disease risks in national herds. This will require substantial investment in managing the biosecurity risk at the processing stage – post farm gate. The creation of co-operatives would ensure that economic benefits accrue to the whole of the farm to fork or fork to farm chain, but particularly to the livestock producers. By this means there will be considerable incentives to continually improve disease status (both production gains and process savings). This is not an untried philosophy, as there have been some successes already in Kenya and Ethiopia; however such programmes will require both the will and the resources to be successful.

A PDF copy of book of synopsis is available online at <u>http://www-naweb.iaea.org/nafa/aph/BookOfExtendedSynopses.pdf</u> or a hardcopy on request.

A sample of the various comments of appreciation from symposium participants

"I wish there was a better word than "Thanks" to express my appreciation for being awarded a grant to participate in the symposium". *Su Su Kyi*

"Congratulations!!!! I would like to thank and congratulate you and the colleagues at the APHS for the very well-organised, prepared and successful Symposium. It was really an excellent one in all parameters". *Moutaz Zarkawi*

"I would like to renewal all my grateful for my invitation in the symposium but also in my participation in the CRP RVF meeting. Many thanks to Gerrit and all his administrative staff". *Lo Modou*

"Again well done on a great meeting!!!" Martyn Jeggo

"I enjoyed working with you - keep up the good work". Ellen Fraser

"Thanks for organizing a very useful symposium. It was indeed a great success and you and your team deserve all the praise for this. Congratulations". *Harinder Makkar*

"Outstanding conference!!!. Thank you also for the opportunity you granted me to chair some of the sessions". *EK Baipoledi*

"Many thanks for these nice days in Vienna, for the good discussions, the constructive symposium and the fantastic hospitality". *Sandor and Kati*

"Thanks indeed for the giving me a chance to visit IAEA and have a meeting with my colleagues from other countries and organizations and share an idea". Z. Batsukh

"I was very glad to get to the symposium last week -and congratulations on the success". *Keith Sumption*

"Thanks to all of you for giving me the opportunity to attend the symposium" Mamadou Niang

Coordinated Research Projects

Development and Use of Rumen Molecular Techniques for Predicting and Enhancing Productivity (D3.10.24)

Technical Officer: E. Nicholas Odongo

The final Research Coordination meeting was held from 8 to 13 June 2009 in conjuction with the APHS symposium. A summary can be found under 'past events' in this Newsletter.

Gene-based Technologies in Livestock Breeding: Phase 1: Characterization of Small Ruminant Genetic Resources in Asia (D3.10.25)

Technical Officer: Mario Garcia Podesta

The final Research Coordination meeting was held from 8 to 13 June 2009 in conjuction with the APHS symposium. A full report can be found under 'past events' in this Newsletter.

Veterinary Surveillance of Rift Valley Fever (D3.20.23)

Technical Officer: Gerrit Viljoen

The main objectives are the evaluation, validation and implementation of rt-PCR and PCR sequencing procedures for early and sensitive detection of the RVF virus and its use in molecular epidemiology using isotopic techniques to improve diagnostic sensitivity (via isotope incorporation into PCR amplicons) and to confirm diagnostic specificity (via hybridization of amplicons with isotope labeled probes). In laboratories equipped with real-time PCR capabilities, the manual PCR procedures are being adapted to include their use as part of the Standard Operating Procedures (SOPs). Manual isotope based slab PCR-sequencing procedures are implemented (In laboratories equipped with automated sequencing equipment, these procedures will be adapted for use). The specific objectives are:

- Evaluation, validation and use of iELISA formats to detect virus-specific antibodies.
- Evaluation of recombinant antigens for use in indirect and competition ELISA's.
- Harmonization of Standard Operating Procedures (SOPs) and introduction of quality assurance procedures for RVF-ELISA.
- Evaluation, validation and implementation of classical rt-PCR and real-time PCR, and PCR sequencing procedures for early and sensitive detection of the RVF virus and its use in molecular epidemiology
- Setting up of a serological and molecular epidemiological database (based on antibody prevalence and virus isolate genetic variation).

This CRP is under the IAEA Project 'Molecular Technologies for Improving Productivity in Smallholder Livestock'. Rift Valley fever (RVF) is one of the several important diseases of livestock that are targeted under this project. RVF epidemics occur at irregular intervals in Africa when heavy rains facilitate the breeding of the mosquito vectors.

Progress towards achieving the objectives of the CRP is satisfactory and all the RCH are within the timeframe of their work plans. In short: The IgG and IgM ELISA platforms (using irradiated virus antigens and control sera) were evaluated, validated and implemented in RCHs laboratories (and other laboratories); The serological procedures were harmonized and are available as SOPs; the RCHs were trained in molecular technologies and quality assurance management; the recombinant RVF antigen was evaluated and is under validation as substitute antigen for the ELISA platforms and epidemiological and surveillance (including sampling frame) strategies were developed and are implemented by all RCHs. The future objectives to be achieved (2009) are: evaluation, validation and implementation of the molecular diagnostic platforms and procedures and their presentation to OIE, FAO and WHO; the validation of the recombinant ELISA platform; the finalization of the DNA and sera reference material; continued maintenance of the established epidemiological databank.

Rift Valley fever (RVF) inflicts great economic losses due to reduced productivity in livestock, widespread abortions in pregnant animals and mortality in young animals. In addition, RVF is zoonotic and may cause debilitating encephalitis, blindness and deaths in humans. The virus was first isolated in 1930 in sheep in the Rift Valley of Kenya and is endemic to sub-Saharan Africa with sporadic outbreaks in the Arabian Peninsula. RFV outbreaks have severe consequences to trade in the region.

RVF epidemics occur at irregular intervals in Africa when heavy rains facilitate the breeding of the mosquito vectors. The latest major outbreaks were in Kenya (2006/7), United Republic of Tanzania (2006/7), (2007/8) and South Africa (2008), leading in some cases to great losses in animals and humans. With rising or fluctuating global temperatures, RVFV can spread to new ecosystems. In September 2000, RVF was first reported outside Africa in Saudi Arabia and Yemen, disrupting all livestock trade from the horn of Africa to the Arabian Peninsula. In Yemen, the 2000 **RVF** outbreak affected more than 2000 humans, killing nearly 300 people, while 20 000 abortions occurred in livestock. This expansion in the epidemic area to the Arabian Peninsula raises the possibility of RVF spread to other parts of Asia and

Europe, especially since RVFV can be spread by a wide range of mosquito vectors. Although transmission is mainly by mosquitoes, it can also occur via contact with infected animals (in the case of veterinarians or abattoir workers), infected blood or tissue samples (laboratory workers) and patients (family, physicians or nursing aides).

Inactivated and attenuated RVF-vaccines are available for veterinary use although both have limitations (they cause abortions), but none are available commercially for humans. It is therefore critical to confine and control the spread of the virus and to limit its spread to non-infected animals and to humans, and prevent spread to non-endemic areas. This can be achieved by the rapid, early and definitive detection of RVFV and consequently controlling animal movement, instituting quarantine measures and/or implementing suitable vaccination strategies. Hence, considerable efforts have been made recently to validate and implement techniques for the rapid and early diagnosis and characterization of RVFV. Enzyme linked immunosorbent assays (ELISA) have the potential to detect RVF specific antibodies in RVFV -infected animals, although their use has been limited due to the lack of standardized procedures, validation data and the unavailability of safe antigens. It is, however, not able to differentiate between vaccinated and field-infected animals; therefore the laborious monitoring of sentinel animals in herds in endemic areas is still required.

In addition, it is of paramount importance to identify the presence of virus as early as possible, even within the window period prior to the development of RVFV antibodies, to allow for the timely implementation of action. Thus the nuclear and nuclear related molecular detection of RVFV was developed and evaluated with a limited panel of field samples. This approach is not only highly specific but can detect presence of RVFV nucleic acid in infected animals during the window period prior to sero-conversion. Only a few of the participating countries were able to implement this technique during this stage and it is expected that all counterparts will participate in the validation phase.

The validation of the serological IgM and IgG platforms (using irradiated or recombinant antigens and irradiated control sera) together with the early stage molecular diagnostic techniques greatly contributed, in the countries experiencing RVF outbreaks (e.g. Kenya and Sudan), to the early and rapid diagnosis of RVF and allowed for the timely response and effective control. The molecular diagnostic technologies and the molecular characterization (i.e. sequencing) of all RVFV isolates will form the last part of this CRP (as basis of the DNA database), using either isotope or fluorescent labeling of sequences, to obtain a genetic databank for the rapid bioinformatical analysis and classification of all isolates. This will allow for molecular epidemiological studies to determine the origin and spread of the virus, the origin of outbreaks as well as in helping to differentiate vaccine virus from wild-type field strains.

There is no human vaccine available, and the animal vaccine that is being used cause abortions in pregnant animals. Two of the RCHs are currently evaluating the efficiency of irradiated RVF as save and potent immunogens (i.e. providing a more complete MHC class I and II protection) for animals and possible humans (outside of the CRP scope).

The final Research Coordination meeting was held from 8 to 13 June 2009 and a full report can be found under 'past events' in this Newsletter.

The Control of Contagious Bovine Pleuro Pneumonia in Sub-Saharan Africa (D3.20.24)

Technical Officer: Hermann Unger

As the publication of the results from the Zambian exercise is now ready for publishing, the CBPP c-ELISA seems to be the tool of choice at the moment. The evaluation of the performance of the test a ring trail was initiated by F. Thiaucourt; the kits and evaluation reagents were ordered and will be distributed soon to 28 MS. The results of this ring trial should be published end of this year and should help improving the laboratory capacities where needed.

The development of a CBPP Loop-mediated Amplification PCR still creates problems. A new experimental setup now for the first time gave good positive results, but there is a need to extend the number of samples. So the traditional PCR's and qPCR will be employed in the near future in the project.

Agreement was reached for the basic evaluation of newly discovered antigens from 2 institutions for the development of a new indirect ELISA. Until end of 2009 experimental "kits" will be available to test the new antigens in the field.

The Early and Rapid Diagnosis of Transboundary Animal Diseases such as Avian Influenza (D3.20.25)

Technical Officer: John Crowther

The IAEA is supporting Member States in their efforts to control diseases of importance. This, amongst others, involves the development, evaluation and validation of the appropriate nuclear and nuclear related technologies and the harmonization of protocols and procedures. Technical advice is therefore given to Member States (or any other party) as to the diagnosis of a disease, the best fitness for purpose tools and quality assured procedures, including vaccines, to use in close collaboration and consultation with experts in the field. In the case of avian influenza it is important for the rapid and differential diagnosis to classify isolates as highly pathogenic or not in order to activate appropriate control measures - this is seen as the bottleneck activity for most developing countries. Highly pathogenic avian influenza (HPAI) now commonly known as bird flu is caused by the infection with some strains of Influenza A virus. The different strains of this virus are classified into subtypes on the basis of their two external proteins named haemagglutinin (H) and neuraminidase (N). Techniques that are implemented for the diagnosis of avian influenza aimed at demonstrating first the presence of the causal virus in pathological samples and then at assessing it's pathogenicity. Indeed, only some strains of avian influenza, highly pathogenic (HPAI), are at the origin of outbreaks and: they belong to the H1, H5 or H7 subtypes. The ongoing avian influenza outbreak which started in Asia in 2004 is caused by a virus of H5 subtype. In addition, this virus was further characterised as of the N1 subtype which is able to cause deaths in humans.

Usually, from the pathological sample, the virus is first isolated in embryonated fowl eggs which takes 4-7 days to complete. Then the subtype of the isolated virus is identified by a battery of specific antibodies raised against the different H (H1 to H15) and N (N1 to N9) proteins. This way of identification is carried out only in specialized laboratories. To confirm a subtype's pathogenicity, the isolate is then inoculated into 4-8 week-old susceptible chickens. For the World Organisation for Animal Health (OIE), strains are considered to be highly pathogenic if they cause more than 75% mortality in inoculated chickens within 10 days. An alternative way to demonstrate the presence, and characterize the influenza virus in the pathological samples, is the specific detection of its RNA by nucleic acid amplification techniques (PCR and PCR sequencing, using either fluorescent or isotopic $[P^{32}, P^{33} \text{ or } S^{35}]$ markers). This molecular approach takes 1-2 days to complete. Furthermore, it is foreseen that this technology could be applied as early warning tools.

The CRP focus on methods which give the best possibilities for earliest detection/diagnosis/confirmation of pathogens or disease. This include "carrier state" animals and newly infected animals (before the development of specific antibodies) and will contribute to the timely response to potential harmful introduction of TADs. It is evident that avian influenza (AI) is a veterinary problem and consequently should be addressed at the animal level. This CRP should widen the possibilities for partners from institutions and industry to enable the research and links with possible 'donors' of technologies to be established. Essentially the CRP involves applied research, wherever possible validating existing formats and evolving working validated protocols for direct detection of AI; differentiation of AI and reporting of the result from the field. The use of nuclear technologies introduces a high level of sensitivity and specificity. In specific, rapid, sensitive and specific detection of disease agent nucleic acids using molecular technologies (e.g. reverse transcription polymerase chain reaction (RT-PCR) and PCR sequencing), applying the use of isotopes $(P^{32/33}, S^{35} \text{ and } S^{35} \text{Met})$ to label PCR amplicons during development and comparative

phases of research, and for the evaluation or characterization of targeted genes.

This CRP is contributing to the Agency's project E2.02, Technologies for reducing risk from transboundary livestock diseases and those for veterinary public health. The overall objective is to develop, evaluate and validate early and rapid detection technologies to provide Member States with the capacity to detect, monitor, contain and control transboundary animal diseases (TADs). The CRP supports the build up of competence in the use of modern biotechnology, including molecular and serological methods, to provide systems and technologies to be used in the field as well as in laboratories. A major target for diagnostic systems will be the highly pathogenic avian influenza (HPAI) viruses, but such systems will be pertinent to all other TADs (e.g. the current Influenza A H1N1 outbreak) since the technologies addressed in this CRP will form part of an early response diagnostic capability platform.

The Early and Sensitive Diagnosis and Control of Peste des Petits Ruminants (PPR) (D3.20.26)

Technical Officer: Hermann Unger

Finally the LAMP PCR for PPR is taking shape. Results obtained so far indicate, that a useful primer set was found and due to the experience gained with avian influenza the production of an experimental LAMP kit is in progress. The real time PCR for PPR developed in Seibersdorf is working fine and after a trial by G. Libeau to verify the results, the reagents should be made available to the participants for field testing. Good results with traditional PCR were already obtained by a number of participants and the published protocol for blood sample collection using filter paper was extended to nasal swabs, giving useful results as well. The competition ELISA works in most laboratories, but some kits did present problems and these should be discussed at the next RCM.

Planned CRP

Genetic Markers and Genetic Variation on the Control of Resistance to Infectious Diseases in Small Ruminants for Improving Animal Productivity

Technical Officer: Mario Garcia Podesta Background:

Small ruminants, mainly sheep and goats, constitute an important livestock resource in most developing countries and are essential for the livelihood of millions of small farmers. Infectious diseases, such as gastrointestinal nematodes impose severe constraints on animal production in pastoral systems worldwide. Losses occur through mortalities, reduced production due to sub clinical diseases and direct costs associated with pest control. Widespread and indiscriminative use of drugs to alleviate parasite infestation has resulted in the emergence of resistant strains in many cases. There are also increasing environmental concerns about chemical residues in meat, milk, and pasture resulting from the use of these drugs.

In many cases disease resistance is a heritable trait and therefore has a genetic basis. This offers the opportunity to select animals for enhanced resistance to the disease. The feasibility of these approaches has been experimentally demonstrated and in other cases, breeding programmes selecting commercial animals for enhanced resistance are being successfully established, especially for sheep as compared to goats. There are concerted efforts to find genetic markers associated with resistance to infections, potentially allowing selection for increased resistance in the absence of infection, especially for scrapie and nematode parasitism.

Biodiversity of small ruminants such as goats and sheep is often expressed in well-adapted traits that enable them to survive harsh, local environmental conditions or show resistance to endemic diseases where other, exotic breeds cannot thrive. Unfortunately, since such traits are not sufficiently characterized, they are underutilized in conventional breeding programmes and there is insufficient research on the ways to select breeds or individuals carrying the most advantageous traits. The characterization and mapping of genes controlling such traits –quantitative trait loci (QTL) and the subsequent use of this information in selection and breeding programmes, should make it possible to facilitate significant increases in small ruminant productivity.

Overall Objective:

Improve productivity in smallholder livestock systems using gene based and related technologies.

Specific Objectives:

- To monitor production and reproduction traits of selected parents based on disease resistance, generating populations suitable for molecular genetic studies.
- To identify genetic markers associated to disease resistance suitable to be used in molecular diagnostics and assisted breeding, either by high throughput SNP panels available or assessing SNPs in candidate genes.
- To develop or validate simple diagnostic tests (PCR, PCR-RFLP, Southern Blot, and short sequences) for identifying animals for breeding purposes.
- To monitor production and reproduction performance of selected animals.
- To develop capacity in developing countries in the use of molecular and related technologies

Nuclear Component:

- Use of radiolabeled nucleotides in DNA hybridization, DNA characterization, and hybrid mapping procedures.
- Use of southern blot technique with radioactive $[\alpha-32P]$ ATP labeling for genetic marker analysis.
- Use of Whole-genome radiation hybrid panel for goats.
- Use of 32P, 35S, Met-35S, 125I immuno-assays in the monitoring of production and reproduction parameters of selected breeds and individuals.

Action Plan

Scientists interested in participating in this CRP may contact us (Mario Garcia Podesta, M.Garcia-Podesta@iaea.org) for specific details of the programme and modalities of participation.

General information applicable to all Coordinated Research Projects

Submission of Proposals

Research Contract proposal forms can be obtained from the IAEA, the National Atomic Energy Commissions, UNDP offices or by contacting the Technical Officer. The form can also be downloaded from the URL http://www-crp.iaea.org/html/forms.html

Such proposals need to be countersigned by the Head of the Institutions and sent directly to the IAEA. They do not need to be routed through other official channels unless local regulations require otherwise.

Complementary FAO/IAEA Support

IAEA has a programme of support through national Technical Cooperation (TC) Projects. Such support is available to IAEA Member States and can include additional support such as equipment, specialized training through IAEA training fellowships and the provision of technical assistance through visits by IAEA experts for periods of up to one month. Full details of the TC Programme and information on how to prepare a project proposal are available at the URL http://www-tc.iaea.org/tcweb/default.asp

For further information contact Svetlana Piedra Cordero (S.Piedra-Cordero@iaea.org)

Activities of the Animal Production Unit (APU) at the FAO/IAEA Agriculture and Biotechnology Laboratory

Development of a Real-Time PCR for the Detection and Quantification of Peste Des Petits Ruminants Virus

Nucleic acid-based detection methods are powerful techniques for specific and rapid detection of pathogens. Currently, PPRV diagnosis based on this technology relies on a gene amplification technique using a classic PCR. For this purpose, the target proteins of the PCR are the nucleocapsid (N) or fusion (F) protein genes. The classical PCR, however, has several limitations of which the most important is the post amplification processing of the products with its concomitant high risk of contamination. This has driven scientists to seek alternative methods and there has been increasing focus on the development of assays relying on the closed vessel systems involved in real time PCR.

A real time PCR based on a classical TaqMan approach has already been described for the detection and quantification of PPRV. It uses the standard 3' quencher fluorophore 6-carboxy-tetramethyl-rhodamine (TAMRA) to quench the 6-carboxyfluorescein (FAM) signal. At APU, we are developing a real time PCR for the detection of PPRV based on the use of a duallabeled fluorogenic probe approach using TaqMan and dihydrocyclopyrroloindole tripeptide (MBG). Since in this method, a non-fluorescent quencher (NFQ) is used in the 3' position instead of the TAMRA and because the MBG allows the design of shorter probes, the fluorescence quenching is more efficient, thereby increasing sensitivity to the assay The amplification primers and the probe used in this test target the virus N gene. Since PPRV isolates are grouped into four discrete lineages, the design of the primers and probe was driven by taking into consideration results from partial N gene sequencing obtained from a representative isolate of each group. Accordingly, they are expected to allow amplification of any PPRV strain.

Preliminary evaluation showed that all four lineages of PPRV viruses could be detected. The assay linearity was determined by amplifying 10-fold serial dilutions of a plasmid containing the PPRV Nigeria vaccine strain (Figure 1). The dynamic range of the assay was found to be of 6 orders of magnitude (107 to 102) with an efficiency of the amplification of -3.25 (R2 = 0.998) as indicated by the slope of the standard curve obtained by plotting the Cycle threshold (Ct) values against the Log10 of the known input copy number (Figure 2). The limit of detection is below 10 copies; even 1 copy / PCR vial can be detected (results not shown). This test also has the advantage of being very fast as only a maximum of 40 cycles is needed (55 minutes with Bio-Rad CFX96).

Further evaluation will involve: -

- Checking test specificity by comparing DNA or cDNA from other pathogens infect ruminants.
- Determining quantitatively the limits of detection by probit regression analysis.
- Validating the assay by using a larger number of isolates of PPRVs from each of the four lineages.



Figure 1: Detection of PPRV by TaqMan MGB real time PCR. The amplification plots were realized on 10-fold serial dilutions (10^7 to 10^2 copies) assayed in triplicate using a plasmid containing the PPRV N gene.



Figure 2: Linearity of the PPRV TaqMan MGB assay. The standard curve was generated by plotting the Cycle Threshold measured in triplicate during three separated runs against the Log of the input copy number.

Diagnosis of Contagious Bovine Pleuro Pneumonia

A new serodiagnostic test has been developed in APU based on an Enzyme Linked Immunosorbent Assay (ELISA), using as coating antigen lipoprotein LppQ, which is specific to Mycoplasma mycoides subsp. Mycoides SC and does not therefore cross-react with other Mycoplasma species. Lipoprotein LppQ was expressed in E. coli identified and characterized by Western blot analysis (WB) and then purified using magnetic beads which bind to the his-tag of the protein. The purified antigen was used for coating microtitre plates to develop a prototype indirect ELISA (iELISA) for the detection of antibodies against CBPP in cattle. After a number of different brands of microtitre plates with different surface reactivity was tested using the purified antigen and standard ELISA protocols, Immulon 1B (Dynatech) plates were identified as being able to produce optimal adsorption of the antigen in the comparative tests. These initial studies confirmed that in order to optimize test parameters it was necessary to effect a reduction in background colour development. This therefore became a priority in the most recent work, aiming to reduce background "noise" due to unspecific binding, thereby enhancing the Binding Ratio (B/B0) and enabling more precise discrimination between positive and negative sera.

A number of commercial blocking buffers were used either non diluted, (Svanovir, Sigma B6429) or diluted in PBS Tween 20 (PBS-T) at different concentrations (Fish Gelatine Amresco at 1% and 5%, Roche Blocking Reagent 5% and Trehalose 5%) these buffers were compared with each other and PBS-T with skimmed milk (Fluka) at 5% and 10% (PBS-T-F5% and PBS-T-F10%) to see which was best suited to achieve a reduction in background. The antigen was coated at a dilution of 1/100 in phosphate buffered saline (PBS), the test serum samples were diluted 1/600 in a blocking buffer, and Horseradish Peroxidase (HRP) was diluted at 1/30000 dilution also in the homologous blocking buffer. The antigen coating stage and the serum and HRP incubation steps were carried out at 37°C for 1 hour in an orbital ELISA shaker. After substrate incubation (TMB/H₂O₂) at 37°C for 15 minutes the reaction was stopped with 1M H₃PO₄ and the optical density (OD) read at 450nm. Five of the buffers were rejected, (Fish Gelatine Amresco 5%, Sigma B6429 (1x), Roche Blocking Reagent 5%, Trehalose 5% and PBS-T-F5%), because they failed to meet the criteria for enhancing the binding ratio. The other three buffers were re-tested and the one that fulfilled the test criteria most completely was selected.

Skimmed milk in PBST at 10% reduced the total OD value of the reference high positive control serum (C++) drastically when used as a diluent for serum and HRP. When the PBS-T-F 10% was used for serum blocking the B/B0 ratio improved but the OD of the reference negative control serum (C-) was still too high (Figure 1).



Figure 1: Example of blocking effect using two different buffers, Svanovir and PBST-F10%.

Optimal binding capacities could be realized with the buffer from Svanovir; but the desired blocking effect was achieved only if the buffer was used for dilution of both serum and conjugate. If the buffer was used as serum dilution only, no blocking effect was observed. (Figure 2).



Figure 2: ELISA checkerboard titration of different positive and negative reference sera versus different conjugate dilutions with Svanovir blocking buffer.

Fellows and Interns at APU

Brigitte M'buze Balobake-so from the Veterinary Laboratory of Kinshasa in Democratic Republic of Congo, is on three months fellowship training at the Animal Product Unit (IAEA Laboratories, ABL). Her project is upgrading laboratory services for diagnosis of animal diseases. The training she gets at the Agency allows her to gain knowledge and experience in the production of monoclonal antibodies.

Anahita Daryabeigi is a graduate of Biology from Azad University of Islamic Republic of Iran. She is taking further studies at the University of Vienna in the field of genetics and microbiology. She had her Diploma thesis at the institute for Cancer Research Vienna, where she mainly focused on Hepatocarcinogenesis. Violeta Dicusara is a Veterinary Medicine graduate from the State Agrarian University of Moldova. She is on her third year in PhD. She has five years of laboratory experience in virology and molecular biology technique.

Christian Schwarz obtained his degree in Ecology and Conservation Biology at the University of Vienna with focus on freshwater and microbial ecology (Masters Degree). He also has a bachelor's degree in Chinese Studies. He worked as a scientific assistant at the University of Vienna, on the role of food quality and its influence on bacterial communities.

IAEA Collaborating Centre on Animal Genomics and Bioinformatics

The IAEA designated in 2004 the Centre on Animal Genomics and Bioinformatics (AGB) as an IAEA Collaborating Centre. This body is composed by four laboratories from three world class research and/or teaching Brazilian institutions [Animal Biotechnology Laboratory (ABL), University of São Paulo, Piracicaba; Laboratory of Molecular Morphophysiology and Development (LMMD), University of São Paulo, Pirassununga; Laboratory of Molecular Biology of Trypanosomatids (LMBT), Oswaldo Cruz Foundation, Rio de Janeiro; and Animal Biochemistry and Molecular Biology Laboratory (LBBMA), São Paulo State University, Araçatuba.

In the first four years of the agreement (2005-2008), the AGB Collaborating Centre has worked, in close consultation with the Agency, on the following programmes:

Assistance to the Agency's training programme through hosting individual fellowship and co-organizing regional training courses such as EMBO World Practical Course on Comparative Genomics, and Molecular biology techniques applied to animal production.

Development, application, and evaluation of new technologies. Among these, the set up of SNP diagnostic kits and performing DNA sequencing services in support of the CRP Gene-based technologies in livestock breeding: Characterization of small ruminant genetic resources in Asia. Besides, in other activities as "Development of real-time PCR tests to detect SNP markers in sheep and its application on breeding and selection schemes for health and production", "*In silico* prospection and standardization of new DNA probes for embryo pre-implantation diagnosis in cattle and sheep", and "Application of selective genotyping and DNA chip technology for detection of divergent alleles for milk production in a composite Holstein and *Bos indicus* cattle".

Collection and dissemination of Information. The Centre has been involved in the preparation, testing (following quality control standards) and delivery of PCR primers and related reagents to Member States, to be used, under supervision of the IAEA, in international, regional, and national genetic studies and breeding schemes. Also, in the coordination of the IAEA "Technical Workshop to Define Breeding Strategies for South American Camelids", in Lima, Peru, in June 2006, with the participation of over 60 participants from Andean countries (Peru, Bolivia, Ecuador, Chile, and Argentina).

The professional staffs of the IAEA Collaborating Centre on Animal Genomics and Bioinformatics has published more than 70 papers in international scientific journals in this period. Among them, can be highlighted the paper "Genome-Wide Survey of SNP Variation Uncovers the Genetic Structure of Cattle Breeds" recently published in Science by The Bovine HapMap Consortium (*Science* 324: 528-532, 2009), in which Dr. Fernando Garcia, the leader of AGB has actively participated.

The IAEA has recently awarded a Technical Contract to the IAEA Collaborating Centre to strengthen partnership and research outputs during the next three years. Activities under this project will be in relation to the development and maintenance of an Internet-based application to liaise the specific participant laboratories (USP, UNESP, and Fiocruz) with the correspondent sectors of the IAEA (especially those related to its major activities in the use of nuclear and molecular tools for animal production improvement, as for instance the Animal Production and Health Section, Animal Production Unit - Seibersdorf, and the Technical Cooperation Department), in order to provide updated tools and information to Member States, research laboratories and centres (e.g. CGIAR), and to the international community in general on the fields of Genomics and Bioinformatics, such as laboratory protocols, standard operating procedures (SOPs), nuclear and related techniques, methodologies and procedures, detailed genome search and analysis tools, radiation hybrid map information, livestock molecular markers database, and discussion forums. All aspects related to the IAEA mandate on the use of nuclear energy for research and development, and on the use of practical applications for improving animal productivity and benefiting mankind will be highlighted and encouraged.

Technical Cooperation Projects

TC Project	Description	ТО
ANG/5/007	Improvement and Veterinary Assistance to Local Small Stock Breeds Objective: The sustainable improvement of small-scale livestock production systems.	Viljoen
BEN/5/003	Veterinary Drug Residue Monitoring Programme Objective: To develop a capacity for veterinary drug residue monitoring in livestock products.	Viljoen Cannavan Patel
BEN/5/006	Improving Animal Health and Productivity Objective : To strengthen, diagnose, and control African swine fever, and increase animal productivity.	Schaten Luckins
BKF/5/006	Establishment of Feeding Tables for Feedstuffs that are Locally Available to Stockholders in Burkina Faso Objective: To improve the reproductive performance of local livestock bred through food supplementation strategies, develop feeding table for locally available food resources, characterize genetic types of cattle used for milk production, improve the effectiveness of artificial insemination on local cattle breeds, and train a qualified team on animal production (nutrition, feeding, reproduction and genetics).	Garcia Podesta Odongo
BKF/5/008	Strengthening the Development of Small Ruminant Production Objective: To combat poverty in the rural environment in Burkina Faso by improv- ing production by evaluating the productivity of different genetic types of small ruminants, improving productivity and reproduction performance of local small ruminants through improved feeding and management practises, and evaluating the impact of gastrointestinal and reproductive diseases in small ruminants and the effec- tiveness of the medicinal plants commonly used by breeders.	Unger Garcia Podesta
BOL/5/019	Implementing Molecular Techniques to Upgrade the Diagnostic Facilities of National Animal Health Programmes (Not funded) Objective : To strengthen the diagnostic capacity of the animal health laboratories supporting programmes for the control and eradication of animal diseases in Bolivia through the use of molecular diagnostic techniques and training of staff in the use of the techniques; to provide rapid and precise diagnosis of animal diseases to allow better control of economically important diseases of livestock.	Crowther/ Luckins
BOT/5/005	Improving Diagnosis of Animal Diseases Objective : To employ nuclear molecular diagnostic techniques for improved diagno- sis of trans-boundary animal diseases, such as foot and mouth disease, contagious bovine pleuropneumonia, and avian influenza.	Viljoen
BUL/5/012	Developing and Validating Molecular Nuclear Technologies for Rapid Diagnostics of Foot and Mouth Disease and Genotyping of Indigenous Cattle Breeds Objective : To improve livestock by rapid diagnosis and effective control of foot and mouth disease, and genotyping of indigenous cattle breeds through development and validation of molecular nuclear methodologies.	Viljoen
BZE/5/004	Strengthening the Veterinary Diagnostic Laboratory with Capacities in Polymerase Chain Reaction Diagnosis (Not funded) Objective : To ensure food security through early detection of H5/H7 Avian influ- enza, and other exotic diseases, and to ensure the capacity for quick response to disease outbreaks with epidemiological surveillance.	Viljoen
CAF/5/002	Assistance for Epidemiological Surveillance of Animal Diseases Objective : To strengthen the diagnostic capacity of the Central Veterinarian Labora- tory (LACAVET) to monitor and control major animal diseases.	Unger

TC Project	Description	ТО
CAF/5/004	Improving Livestock Production Through Disease Control and Artificial Insemina- tion	Unger Garcia Podesta
	Objective : To improve animal production in the Central African Republic through livestock disease control and improved breeding by use of artificial insemination.	
CMR/5/015	Use of Nuclear Techniques for Improving Ruminant Productivity & Disease Control Objective : Develop capability for improved breeding by disease control and artificial insemination.	Garcia Podesta Unger
CMR/5/017	Improving Animal Productivity and Health Objective : To strengthen capacity and outreach regarding artificial insemination in ruminants, and to control livestock diseases impeding reproduction and productivity.	Unger
ERI/5/005	Zoonotic (diseases that can be transmitted from animals to humans) Disease Control and Analysis of Veterinary Residues in Foods Objective : The objective of the project is to determine: 1. The epidemiological prevalence of brucellosis and tuberculosis in the major dairy producing areas; 2. Baseline data on veterinary drug residues in milk and meat products.	Patel Unger
ERI/5/006	Controlling Major Epizootic Diseases and Other Mycoplasma Infections of Livestock Objective : To improve the control of transboundary animal diseases, and continue the eradication of tuberculosis and brucellosis.	Patel Unger
ETH/5/012	Integrating Sterile Insect Techniques for Tsetse Eradication Objective : To eradicate the tsetse fly from the southern Rift Valley, thereby creating an environment conducive to livestock development and improved agricultural pro- duction.	Feldmann Parker Viljoen
ETH/5/014	Monitoring and Control of Major Animal Diseases Objective : To strengthen the diagnostic capacity of the National Veterinary Institute to monitor and control trans-boundary diseases, particularly foot and mouth disease and contagious bovine pleuropneumonia.	Viljoen
GAB/5/002	Diagnosis and Control of Animal Diseases Objective: To aid identification and control of livestock diseases.	Crowther/ Luckins Unger
GHA/5/033	Control of Animal Pests that Affect Small Ruminants(Not Funded) Objective: To determine the efficacy of the PPR vaccine currently used in Ghana	Unger
HON/5/004	Improving the Nutrition and Health Conditions of Livestock in Order to Increase Productivity and Reproductivity (Phase II) Objective : To strengthen and improve livestock production in Honduras.	Garcia Podesta Odongo Viljoen
HON/5/005	Improving the Nutrition and Health Conditions of Livestock in Order to Increase Productivity and Reproductivity (Phase II) Objective: To strengthen and improve livestock production in Honduras.	Garcia Podesta Odongo Viljoen
INS/5/033	Enhancement of Quality Assurance for the Analysis of Veterinary Drug Residues Objective : To enhance the national capacity to ensure the safety of food products of animal origin.	Patel Cannavan
INS/5/034	Development of Environmentally Sound Livestock and Agricultural Production Objective : To improve livestock productivity without adversely affecting the environment through improved feed supplementation strategies, managing nutrient waste on farms and reducing methane emissions.	Odongo
IRA/5/012	Preparation of ELISA Kits for Diagnosis of Foot and Mouth Disease Objective : To establish the ability to prepare standardized assays for use in foot and mouth disease (FMD) control.	Crowther/ Luckins
IVC/5/030	Assessing the Genetic Profile for Improved Livestock Production Objective : To assess the genetic profile of livestock for the effective revival of stockbreeding in Côte d'Ivoire.	Unger Garcia Podesta

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TC Project	Description	то
KEN/5/027	Assessment of Local Feed Resources for Enhancing Fertility and Productivity of Smallholder Dairy Cattle	Odongo Garcia Podesta
	Objective : To assess the potential of local feed resources for enhancing the fertility and productivity of smallholder dairy cattle in the Nakuru District of Kenya.	
KEN/5/028	Applying Nuclear Based Techniques to Control Animal diseases Objective : To improve the capacity to diagnose and carry out surveillance of Conta- gious Bovine Pleuro-Pneumonia (CBPP), Brucellosis, Rift Valley Fever (RVF), Peste Des Petits Ruminantes (PPR) and Highly Pathogenic Avian Influenza (HPAI) using nuclear and related technologies.	Crowther/ Luckins Viljoen
MAG/5/016	Applying Nuclear Techniques to Optimize Animal Production Objective: To increase animal production through the improvement of animal health and control reproduction in the Amoron'i Mania region.	Garcia Podesta Odongo Crowther/ Luckins
MAU/5/002	Improving the National Capacity in Diagnostics for Animal Diseases (Infection and Parasitic Diseases)	Luckins Schaten
	Objective : To strengthen the diagnostic capacity of the Centre National D'Elevage et de Recherches Veterinaires (CNERV) to monitor and control trans-boundary animal diseases, particularly foot and mouth disease and contagious bovine pleuropneumonia.	Senaten
MAU/5/003	Improving the National Capacity in Diagnostics for Animal Diseases (Infection and Parasitic Diseases) Objective : To strengthen the diagnostic capacity of the Centre National D'Elevage et de Recherches Veterinaires (CNERV) to monitor and control trans-boundary animal diseases, particularly foot and mouth disease and contagious bovine pleuropneumonia	Unger Schaten
MLI/5/019	Improving Pneumopathies Diagnosis in Ruminants Using PCR Objective : To improve knowledge about the epidemiology of the dominant respira- tory pathologies affecting small ruminants in Mali's agro-pastoral areas through improving the diagnosis of pneumopathies in small ruminants to support the national control and eradication programme.	Viljoen
MLI/5/023	Improving National Capabilities for Characterization of Serotypes of Major Animal Diseases Using Molecular Biology Techniques Objective : To identify various serotypes present in Mali in order to improve animal health and increase productivity in milk and meat through increased capabilities for diagnosis and control of foot and mouth disease, trypanosomes and tuberculosis.	Unger Viljoen Schaten
MON/5/013	Diagnosis and Surveillance of Transboundary Animal Diseases and Production of Diagnostic Reagents Objective : To obtain international recognition of freedom from several transbound- ary animal diseases, to develop a capacity for the local production, standardization and validation of diagnostic reagents and diagnostic kits, and to establish a quality system for diagnosis of transboundary animal diseases using the local produced diagnostic kits.	Crowther/ Luckins Viljoen
MON/5/016	Improving Productivity of Cattle, Camels and Yaks Through Better Nutrition and Reproductive Management Objective : To increase milk, meat and wool production of yaks, cattle and camels by improving the quality and quantity of feed with high nutritional value and tolerance to low temperature and improving the genetic potential using artificial insemination coupled with radio immunoassay for progesterone.	Odongo Garcia Podesta
MON/5/017	Supporting the Sustainable Production and Supply of Vaccines and Diagnostic Kits for Transboundary Animal Diseases Objective : To produce vaccines and diagnostic kits for transboundary animal dis- eases.	Crowther/ Luckins Viljoen

TC Project Description

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MOR/5/030	Improving Sheep and Goat Production in Morocco through Genomic and Reproduc- tive Physiology Characterization with the Help of Radio-immunoassay and Molecu- lar Techniques (Not yet funded) Objective : Increase sheep and goats for consumption and producers' revenue while preserving natural resources.	Garcia Podesta Malek
MOZ/5/002	Promoting sustainable Animal Health, Reproduction and Productivity Through the Use of Nuclear and Related Techniques Objective : To obtain sustainable improvement in animal reproduction and breeding and animal health through the use of nuclear and nuclear related technologies.	Viljoen
MYA/0/006	Human Resource Development and Nuclear Technology Support Objective : To upgrade and strengthen the skills and capabilities of human resources within the broad range of the applications of nuclear science and technology.	Dias Khan Crowther/ Luckins
MYA/5/013	Integrated Approach for Enhancing Cattle Productivity Objective : To improve smallholder dairy cattle production in Yangon and Mandalay regions.	Garcia Podesta Odongo
MYA/5/015	Strengthening the National Capacity for the Production of Veterinary Vaccines Objective : To enhance the national capacity for quality vaccine production to support efforts to control infectious diseases in livestock production, particularly FMD.	Crowther/ Luckins Cannavan
MYA/5/018	Enhancing the Lifetime Health and Performance of Offspring and Improving the Profitability of Livestock Production Systems Through Selective Breeding and Man- agement of the Maternal Environment Objective : To improve livestock production and thereby increase profitability through improved management of the maternal environment and health care pro- grammes; b) To train technicians in advanced technologies in the field of research and development, breeding, reproduction, dairy production, nutrition and waste management and train technical staff in livestock data analysis and data processing.	Garcia Podesta
NER/5/011	Upgrading Laboratory Services for Diagnosis of Animal Diseases Objective : To support the Government effort in controlling main livestock trans- boundary diseases, mainly contagious bovine pleuropneumonia (CBPP), peste des petits ruminants (PPR) and foot and mouth disease (FMD). To help improve the national animal disease diagnosis capabilities at the Laboratoire Central d'Elevage (LABOCEL) in the use of modern techniques to obtain specific and rapid results with focus to CBPP, PPR and FMD.	Luckins Unger
NER/5/013	An Integrated Approach for Improvement of Livestock Productivity Objective : To increase the productivity of livestock through implementation of an integrated programme dealing with nutrition and reproduction.	Odongo Garcia Podesta Luckins
PER/5/029	Genomics of the Alpaca: Identification of Expressed Genes and Genetic Markers Associated with Productivity and Embryonic Mortality Objective : To identify and characterize the factors associated with embryonic mor- tality in alpacas.	Garcia Podesta Malek
RAF/5/054	Improvement of Livestock Productivity through an Integrated Application of Tech- nologies (AFRA III-4) Objective: To develop and facilitate the application of appropriate selection criteria for genetically improved stock; to institute integrated management, nutrition, health- care and follow-up practices for genetically improved stock; and to use modern reproductive techniques to improve productivity and reproductive efficiency of livestock in the region.	Garcia Podesta Odongo

TC Project Description

RAF/5/055 Support to African Union's Regional Programmes for Control and Eradication of Viljoen Major Epizootics

Objective: To support within the framework of a strategic partnership with the African Union, the global effort of control and eradication of major trans-boundary animal diseases affecting livestock in the region led by the Inter-African Bureau for Animal Resources (AU/IBAR). This programme will aim at helping African countries to improve and produce livestock to ensure their role and participation in international markets that will lead to poverty alleviation and increased livelihoods. The specific objectives of the project are (i) to provide support to selected national veterinary laboratories to implement a quality assured disease control programme; (ii) to transfer appropriate and state-of-the-art technology to support diagnostic, surveillance and epidemiological activities relating to the control of major livestock diseases; and (iii) to support the establishment of a regional centre in Africa (Pan African Veterinary Vaccine Centre [PANVAC]) that would be responsible for (a) the production, assembly and distribution of diagnostic kits; (b) evaluating and monitoring the development of quality assured animal vaccines and (c) advising on the use of vaccines and vaccine strategies.

- RAF/5/057 Strengthening Capacities for the Diagnosis and Control of Transboundary Animal Viljoen Diseases in Africa (AFRA) Unger **Objective:** To strengthen the diagnostic capacity of national veterinary services to monitor and control major transboundary animal diseases, particularly foot and mouth disease, peste des petits ruminants and contagious bovine pleuropneumonia.
- RAS/5/044 Integrated Approach for Improving Livestock Production Utilizing Indigenous Resources and Conserving the Environment (RCA) Odongo Objective: To improve livestock productivity through better nutritional and reproduction strategies while conserving the environment. The specific objectives are to improve animal productivity and decrease discharges of selected greenhouse gases, (methane and carbon dioxide) and selected nutrients (nitrogen and phosphorus) into the environment; and to identify and adopt better breeding strategies that will improve animal productivity through the use of better selection criteria for offspring from cross-breeding programmes, optimum utilization of appropriate indigenous cows, benchmarking for growth and reproduction, and improving procedures for
- RER/5/015 Supporting Early Warning and Surveillance of Avian Influenza Infection in Wild and Domestic Birds and Assessing Genetic Markers for Bird Resistance **Objective**: To establish early bird flu diagnosis and assessment of genetic markers for AI resistance with nuclear molecular methods in the region of Bosnia and Herzegovina, Bulgaria, Croatia, Macedonia, Montenegro, Serbia, Turkey, Uzbekistan, Kyrgyzstan and Russia.

management, nutrition and healthcare programmes in dairy farms.

- RLA/5/049 Integrated Control of Fascioliasis in Latin America (in support of National Programmes
- Improving the Productivity of N'dama Cattle SIL/5/006 **Objective:** To establish a national capability for the application of nuclear techniques to (i) assess the nutritional quality of locally available feed resources, and to develop optimal feeding strategies, (ii) evaluate the reproductive performance under different management and nutritional conditions, and improve artificial insemination (AI) services, and (iii) diagnose and determine epidemiological status of important diseases.
- Improving the Productivity of Ndama Cattle In Sierra Leone SIL/5/010 Objective: To strengthen the diagnostic capacity to monitor and control animal diseases affecting cattle, (ii) to apply feeding strategies and supplementation packages, and (iii) to produce hydrids with greater potential for increased growth rate and milk yields.

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TC Project	Description	ТО
SIL/5/011	Controlling Economically Important Livestock Diseases Objective : To design epidemiological surveys and adopt appropriate rapid laboratory techniques for the diagnosis of PPR and NCD in small ruminants and local chickens.	Unger
SRL/5/041	Maximizing Productivity on Goat Farms through Cost-Cutting and DNA-Based Technology in Selection for Breeding Objective : To improve the productivity of goats of small-holder farmers in Sri Lanka, by introducing new strategies such as supplementary feeding, improved management practices and disease control and by transferring genetic technologies to assist in proper selection of superior breeding animals.	Garcia Podesta Odongo Viljoen Malek
SRL/5/042	Applying Molecular Diagnostics to Zoonotic Diseases Objective : To enhance the long-term epidemic preparedness by developing compe- tence in molecular diagnosis and surveillance of zoonotic infections.	Khan (NAHU) Unger
SUD/5/028	Epidemiology and Control of Snail-borne Diseases in Irrigated Areas Objective : The overall objectives of the project are to increase animal production, and maintain healthy and productive herds in irrigated areas by controlling snail- borne diseases.	Unger
SUD/5/029	The Characterization and Quality Assured Production of an Attenuated Theileria Annulata vaccine Objective : To protect cattle against tropical theileriosis through vaccination in order to improve animal health and reduce reliance on acaricidal/pesticide tick control. More specifically, to establish quality-assured procedures and protocols for T. annu- lata cell culture vaccine production.	Unger
SUD/5/031	Setting up a National Network for the Control of Livestock Diseases that affect Exports Objective : To establish capacity to diagnose Brucellosis in ruminants to improve food safety and secure animal exports.	Unger
TAD/5/003	Diagnosis and Control of Brucellosis in Cattle, Sheep and Goats Objective : To improve diagnosis of brucellosis in cattle, sheep and goats in order to prevent the spread of the disease among animals and the human population in Tajiki- stan.	Crowther/ Luckins
UGA/5/028	Improving the Capacity for Diagnostic of Animal Diseases Objective : To strengthen the diagnostic capacity of the Diagnostics and Epidemiol- ogy Laboratory of the Ministry of Agriculture, Animal Industry and fisheries to monitor and control transboundary animal diseases of importance (e.g. CBPP, FMD, AI, Rabies, Brucellosis and RVF) to Uganda.	Viljoen Unger
UGA/5/030	Improving the Diagnostic Capacity in Animal Diseases (Phase II) Objective : To strengthen the diagnostic capacity of the National Animal Diseases Diagnostics and Epidemiology Laboratory in the detection of animal disease and food-borne pathogens including drug residues.	Crow- ther/Luckins Unger
URT/5/025	Support for the Delivery of Artificial Insemination services Objective : The sustainable intensification of milk and meat through the provision of efficient and reliable AI services.	Garcia Podesta
URU/5/026	Increasing the Profitability of Dairy Producers by Improving Reproduction Effi- ciency, Rational Sustainable Use of Genetic Resources Objective : To implement integrated management strategies to improve the profitabil- ity of medium size grazing dairy farms by means of (a) integrated nutritional strate- gies; (b) strategic reproductive interventions; and (c) marker-assisted selection.	Garcia Podesta Odongo
VIE/5/016	Applying In Vitro Gas Production and Purine Derivative Techniques to Determine the Metabolizable Energy and Protein of Feeds for Beef and Dairy Cattle Objective : To improve the profitability and sustainability of beef and dairy produc- tion through the development of an Net Energy (NE), Metabolizable Energy (ME) and Metabolizable Protein (MP) feeding system for tropical animals and feedstuffs in Vietnam.	Odongo

TC Project	Description	ТО
YEM/5/006	Quality Management for Upgrading Animal Disease Control Objective : To improve the management of diagnostic testing for livestock diseases in Yemen, leading to increased assurance of results in aiding control programmes.	Crowther/ Luckins Viljoen
ZAI/5/015	Upgrading Laboratory Services for Diagnosis of Animal Diseases Objective : Control and eradication of livestock transboundary diseases or other epizootics through the laboratory investigations using nuclear and related technolo- gies.	Unger
ZAM/5/025	Development of Feeding Strategies for Smallholder Dairy Animals in Njolwe and Palabana Dairy Tenant Schemes Objective : To improve household food security and income generation among small scale farmers through increased production and marketing of livestock by developing sustainable feeding and breeding strategies based on increased use of locally avail-	Garcia Podesta Odongo

able resources.

Publications

Recently Published

The Bovine HapMap Consortium published the paper entitled "Genome-Wide Survey of SNP Variation Uncovers the Genetic Structure of Cattle Breeds" in Science (Science 324: 528-532, 2009)

In this paper, more than 90 scientists have substantially contributed under the leadership of three project leaders to analyze the genetic structure of various breeds of cows. They work in a coordinated manner, as project group and breed group leaders, or doing specific activities such as pedigree analysis and breed sampling, sample acquisition and DNA preparation, genome assembly, SNP discovery, ENCODE resequencing, genotyping, database and Web site development, QA/QC, allele frequency analysis, map data provision and analysis, haplotype estimation, longrange LD analysis, LD persistence across breeds, selective sweeps, and on applications. Among this large group of dedicated scientists, we would like to highlight the participation of Dr. Paul J. Boettcher, former IAEA Technical Officer and current FAO Officer, and Drs. Jose Fernando Garcia, Olivier Hanotte, and Paolo Ajmone-Marsan, all agreement holders of the FAO/IAEA CRP on "Gene-based Technologies in Livestock Breeding: Characterization of Small Ruminant Genetic Resources in Asia".

The emergence of modern civilization was accompanied by adaptation, assimilation, and interbreeding of captive animals. However, despite mapping and diversity studies and the identification of mutations affecting some quantitative phenotypes, the detailed genetic structure and history of cattle are not known. Cattle occur as two major geographic types, the taurine (humpless - European, African, and Asian) and indicine (humped - South Asian, and East African), which diverged more than 250 thousand years ago. In this study, individuals representing 14 taurine, 3 indicine and 2 hybrid breeds, as well as few buffalo breeds were sampled. This resulted in a study of more than 37,000 SNPs in nearly 500 individual cows from 19 geographically and biologically diverse breeds. The data showed that cattle have undergone a rapid recent decrease in effective population size from a very large ancestral population, possibly due to bottlenecks associated with domestication, selection, and breed formation. Domestication and artificial selection appear to have left detectable signatures of selection within the cattle genome, yet the current levels of diversity within breeds are at least as great as exists within humans. This paper is the first glimpse that we have to manipulate the genetic makeup of our ruminants (genotypic).

IAEA-TECDOCS are available electronically on: http://www-pub.iaea.org/MTCD/publications/publications.asp Orders for hardcopies and requests for information may be addressed directly to: **Sales and Promotion Unit,** International Atomic Energy Agency, Wagramerstrasse 5, P.O. Box 100,A-1400 Vienna, Austria, Telephone: +43 1 2600 22529 (or 22530); Facsimile: +43 1 2600 29302; E-mail: sales.publications@iaea.org http://www-pub.iaea.org/MTCD/publications.asp

In Press

Managing Prenatal Development to Enhance Livestock Productivity

Responsible Technical Officer: E. Nicholas Odongo The need for a book dealing with managing prenatal development to improve livestock productivity was identified during a Consultants meeting on 'Research Needs for Improvement of Livestock Productivity in Developing Countries through Manipulation of Nutrition in Utero', held in October 2005.

The objectives of this book are to provide a quantitative assessment of the role of, and current state of understanding of the mechanistic basis to, environmental plasticity in producing healthy and productive livestock. The book will contain review papers covering all the key livestock species as well as chapters covering relevant information on non-livestock species.

In vitro Screening of Plant Resources for Extranutritional Attributes in Ruminants: Nuclear and Related Methodologies

Responsible Technical Officer: E. Nicholas Odongo This document is the outcome of a meeting between the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture and Writtle College titled 'Alternative feed resources: a key to livestock intensification in developing countries' held on 14/15 September 2006. The following broad topics will be included in the document:

- Challenges in extrapolating *in vitro* data to in vivo evaluation of plant resources
- *In vitro* screening of feed resources for efficiency of microbial protein synthesis
- Assessing antiprotozoal agents
- Screening plants and plant products for methane inhibitors

- Screening plants for the antimicrobial control of ruminal acidosis.
- *In vitro* methods for screening plant products for direct activity against ruminal gastrointestinal nematodes.

This publication will be of interest to MS wishing to improve livestock productivity, improve utilization of digestible nutrients, reducing the use of drugs and chemicals in livestock production, and reducing environment impact of livestock to meet national and international requirements for trade in food and food products. The target audience/readership include current, and future TCP and CRP counterparts, laboratory managers and staff, fellows and training course participants ay regional and inter-regional courses, enthobotanists, enthoveterinarians, and tertiary training institutions in livestock production and plant secondary compounds.

Veterinary Diagnostic Real-time PCR Handbook

Responsible Technical Officer: Gerrit Viljoen

The uses of nucleic acid-directed methods have increased significantly in the past five years and have made important contributions to disease control country programmes for improving national and international trade. These developments include the more routine use of PCR and real-time PCR as diagnostic tools in veterinary diagnostic laboratories. However, there are many problems associated with the transfer and particularly, the application of this technology. These include lack of consideration of: the establishment of quality-assured procedures, the required setup of the laboratory and the proper training of staff. This can lead to a situation where results are not assured.

This book will give a comprehensive account of the practical aspects of real-time PCR and strong consideration will be given to ensure its optimal use in a diagnostic laboratory environment. This includes the basic principles, setting-up of a real-time PCR laboratory; Good Laboratory Practice and Standard Operating Procedures; Diagnostic Implementation, Execution and Interpretation and Problem Solving. Examples of Standard Operating Procedures and an outline of training materials necessary for real-time PCR technology transfer will be presented. The difficulties, advantages and disadvantages in PCR and real-time PCR applications will be explained and placed in context with other test systems.

Emphasis will be placed on the use of real-time PCR for detection of pathogens, with a particular focus on diagnosticians and scientists from the developing world. It is hoped that this book will enable readers from various disciplines and levels of expertise to better judge the merits of real-time PCR and to increase their skills and knowledge in order to assist in a more logical, efficient and assured use of this technology.

The following major areas will be included in the document:

- Traditional PCR
- Real-Time PCR- The Basic Principles
- Diagnostic Real-Time PCR Applications (e.g., TaqMan, Molecular beacons, Primer-Probe Energy Transfer methods, others)
- Novel PCR techniques aimed for diagnostic use
- Laboratory automation, molecular diagnostics
- Real-time PCR analysis and interpretation/statistical analysis
- Real-time PCR laboratory set-up and quality assurance management of the diagnostic laboratory and the diagnostic test (Including Quality assurance and validation of molecular assays).

This publication will be of interest to the Member States wishing to improve disease control country programmes for improving national and international trade. The recipients will include current, past and future TCP and CRP counterparts, laboratory managers and staff, fellows and training course participants at regional, interregional courses, and tertiary training institutions in livestock production.

Selection and Breeding of Cattle in Asia: Strategies and Criteria for Improved Breeding

Responsible Technical Officer: Mario Garcia Podesta This publication contains the outcome of a Consultants Meeting conducted under the framework of a regional IAEA/RCA project on 'Integrated approach for improving livestock production using indigenous conserving environment' resources and the (RAS/5/044). The need for such a document was identified during the first planning meeting of the project. The topics covered are all relevant to the IAEA programme, including artificial insemintion (AI) nuclear techniques on livestock reproduction and breeding and to the activities being undertaken by project counterparts with support from national and regional projects in Asia. The topics covered include: information about trends in livestock production and cattle breeding management in Asia; the important traits for dairy and beef cattle, their selection criteria, and breeding objectives; proposed systems for operating a cattle breeding and genetic improvement programme in Asia; and an overview of current and future technologies for improvement of cattle breeding.

Instant Testing and Reporting Systems

Responsible Technical Officer: Gerrit Viljoen

A paper is to be submitted on Instant Testing and Reporting Systems to the OIE. The Joint FAO/IAEA Division is actively supporting the system which comprises highly mobile rugged and operator fool proof devices to perform diagnostics with defined diagnostic specificities ad sensitivities which can instantly send results back from the field to a central control point. In this way real-time diagnosis can be made. The devices use variants of the PCR and instant extraction and analysis of samples, thereby avoiding the problems of sample storage and transport- often the most damaging feature to molecular tests. The system is at the heart of the CRP D3.20.25 involving the rapid diagnosis of avian influenza. The devices will be validated in reference laboratories and then in the field. Such systems offer the way forward to improving early warning of disease spread and should revolutionize diagnosis in developed and developing countries.

In Preparation

Integrated Approach for Improving Livestock Production Using Indigenous Resources and Conserving the Environment

Responsible Technical Officer: E. Nicholas Odongo Livestock farming is very important in Asia and the pacific region as a source of livelihood for resource poor farmers, i.e. for provision of food and food products and as a source of income. However, livestock productivity in many countries is below their genetic potential because of inadequate and imbalanced feeds and feeding, poor reproductive management and animal diseases which is exacerbated by lack of effective support services, such as animal husbandry extension, artificial insemination (AI) and/or veterinary services.

The International Atomic Energy Agency (IAEA) and the Regional Cooperative Agreement for Asia and the Pacific Region (RCA), with technical support of the Joint FAO/IAEA Programme of Nuclear Techniques in Food and Agriculture, implemented a Technical Cooperation (TC) project entitled 'Integrated approach for improving livestock production using indigenous resources and conserving the environment' (RAS/5/044). The specific objectives of this project were: (a) to improve animal productivity and decrease discharges of selected greenhouse gases (GHG; methane and carbon dioxide) and selected nutrients (nitrogen and phosphorus) into the environment; and (b) to identify and adopt better breeding strategies to improve animal productivity through the use of better selection criteria for offspring from cross-breeding programmes, optimum utilization of appropriate indigenous cows, benchmarking for growth and reproduction, and improving procedures for management, nutrition and healthcare programmes in dairy farms.

This publication contains research results presented by scientists during the final review meeting. Briefly, the participating MS have developed strategies to reduce methane production and conserve the environment. The feeding strategies have resulted in increased weight gains and milk production in dairy animals. Increased milk yields of approximately 25% were observed in Bangladesh and in the Philippines. Bangladesh, China, Indonesia and Myanmar reported increased average daily weight gains ranging from 15 to 70%. The reduction in methane production due to adoption of the new feeding strategies in Bangladesh, China Indonesia, Pakistan and Thailand ranged from 15 to 70%. Bangladesh, Indonesia, Pakistan and Sri Lanka have also reported increased N and P content of manure which has resulted in 25 to 40% increase in rice and fodder yields. Most of the participating countries have disseminated the knowledge for efficient manure management to end users. Selected farmers (lead farmers) have been trained on the new feeding strategies.

Almost all the participating MS have also achieved genetic improvement in their livestock through different reproductive techniques. For example, India and Sri Lanka through use of synchronization programmes and insemination with genetically superior semen, Bangladesh through IVF and ETT programmes and Myanmar, Philippines, Indonesia, Thailand through crossbreeding programmes. Most of the MS have designed and applied the criterion for selection of better heifers e.g. in India selection is based on weight gain and parents performance while in the Philippines additional parameters like milk composition have been taken into consideration. A laboratory protocol for IVM, proper semen preparation as well as Cryobanking of semen, IVF of IVM oocytes was established in Bangladesh, Sri Lanka and Indonesia. All the participating countries have included improved reproductive techniques and nutritional supplementation strategies to improve production and reproduction of local and crossbred cattle/buffaloes.

The contributions of the experts associated with RAF/5/044 have been incorporated in the report and it is hoped that this publication will help stimulate further research and development into ways of improving the efficiency and productivity of livestock, leading to higher incomes and livelihoods for smallholder farmers.

Economic Impact of Targeted Interventions to Improve Productivity of Peri-Urban Small-holder Dairy Farms

Responsible Technical Officer: Mario Garcia Podesta This document was produced under an IAEA Coordinated Research Project entitled Integrated Approach for Improving Small Scale Market Oriented Dairy Systems, with technical support of the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture. It details the results obtained by project counterparts from interventions to improve animal productivity by overcoming the most important contraints identified during Participatory Rural Appraisals made and Economic Opportunity Surveys performed in direct interaction with stakeholders. The publication presents both the results of case studies in which the interventions were applied and methods for evaluating their economic impact. The publication is intended for livestock specialists involved in the management of dairy production services for cattle farmers in Asia, including those in Ministries of Agriculture/Livestock, Departments of Livestock and Veterinary Services, AI centres, public and private veterinarians and consultants.

CD-ROMs

A CD-ROM is available dealing with training material for the diagnosis of rinderpest and for the preparation for the OIE pathway. It was produced under an IAEA Technical Cooperation project RAF/0/013 ICT based training to strengthen LDC capacity. Contact Gerrit Viljoen at <u>g.j.viljoen@iaea.org</u> for further information. A new batch of CDs with a training package to help artificial insemination (AI) technicians to improve the performance of AI and field services provided to farmers was produced for users with a slow internet connection and is now available through the APHS. It is also accessible from the AP&H Section website: http://www-naweb.iaea.org/nafa/aph/index.html

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Websites

The AP&H Section website is being updated on a regular basis. Please feel free to look at it and make comments <u>http://www-naweb.iaea.org/nafa/aph/index.html</u>



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International Atomic Energy Agency Wagramer Strasse 5, P.O. Box 100, 1400 Wien, Austria

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