

Joint FAO/IAEA Programme Nuclear Techniques in Food and Agriculture

# Animal Production & Health Newsletter

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Brown Swiss heifers well adapted to the high altitude and cold weather on the highlands of Peru

# **To Our Readers**

#### Dear Colleagues,

The year was highlighted by our 'International Conference on Sustainable Improvement of Animal Production and Health' that was held from 8-11 June in Vienna, Austria. It was attended by approximately 400 delegates from 100 Member States as well as representatives of international organizations including FAO, WHO, OIE and ILRI, to present and discuss strategies for the sustainable improvement of animal production and health, particularly the development and implementation of food security and poverty alleviation. Twenty-four keynote speakers focused on the current situation and the ways in which the means for increasing food security might be met. The seriousness with which this topic was perceived by Member States (MS) was shown in the results of their research, presented orally by 53 delegates, together with another 163 poster displays. Crucially, the Symposium provided a forum for robust discussion, fostered exchange of information, identified issues of concern to MS and facilitated ways to develop solutions to the problems using nuclear and nuclear related technologies. It is fair to say that this Symposium was a big success and I thank all involved.



As 2010 is dawning on us, I want to look forward and highlight some of the exciting new nuclear and nuclear related areas that we think will play an important role in the near future. The main constraint to livestock production in many tropical regions of Africa, Asia and Latin America is the scarcity and fluctuations in quality and quantity of the year-around supply of feeds. Animal productivity is restricted by the low nitrogen and high fibre content of the native grasses and crop residues, which form the basis of the diets in these regions. An additional consequence of this poor diet in ruminants is the increased production of methane compared with ruminants fed on better quality forages. Studies on rumen metabolism using isotopes of carbon, hydrogen, sulphur, phosphorus and nitrogen have revealed how the ruminal microbial flora can change depending on the type of forage ingested. In addition, certain plant metabolites reduce the microbial population thereby improving efficiency of feed utilization by up to 10 per cent. The future application of this strategy is that the modulation of fermentation can reduce methane production, hence mitigating greenhouse gas emissions.

The early, rapid and sensitive diagnosis of transboundary animal diseases is a high priority for MS to ensure food security. Most of the modern biotechnologies rely on nuclear inputs, either in their development or their implementation phase. Nuclear, nuclear associated and nuclear related techniques, including enzyme linked immunosorbent assay (ELISA), nucleic acid amplification methodology (polymerase chain reaction, PCR) and PCR nucleic acid sequencing, ensure that these food security priorities are met. Recently developed real time PCR tests for capripox virus infection in small ruminants and cattle now enable specific identification of the causal virus as sheep pox virus, goat pox virus or skin disease virus. Newly developed lumpy immunoassays for Contagious Bovine Pleuropneumonia (CBPP) have also been transferred to MS to undergo validation in the field. There is an advantage in providing diagnostic results at the penside, to enable immediate decisions on appropriate control measures, especially in the case of transboundary diseases, including Highly Pathogenic Avian Influenza, (HPAI) where suspected disease may occur in remote areas. State-of-the-art, loop mediated isothermal amplification tests that can give results within an hour and can be modified for penside use are being developed for detecting identify (HPAI) and human pandemic agent H5N1. Similar technologies are also being applied the diagnosis of the African tsetsetransmitted Trypanosoma congolense and T. vivax and for Peste des Petits ruminants (PPR) virus infection the technology is being developed further to provide dipstick kits. The threat of Foot-and-mouth disease (FMD) outbreaks is constantly with us and we need to address the virus cartography and isolate origin in our vaccine formulations (please see our planned FMD CRP for 2010).

Establishing migratory connectivity in wild birds, that is their place of origin and breeding habitats, is a vital component in the strategy to combat HPAI. Groundwater isoscapes in waters around the globe influence the composition of isotopic hydrogen (\deltaD) in terrestrial food chains. The isotope in water is taken up in the feathers of migratory waterfowl so by analysing and quantifying its presence the isoscape whence the birds came can be identified by correlating the data for isotopic grids of hydrogen constructed using the IAEA's and World Meteorological (WMO) Global Network of Isotopes in Precipitation (GNIP) database with that for feather isotopes. The IAEA is a key player in initiating and coordinating such studies given its current involvement in training and research in the epidemiology of Avian Influenza. The IAEA will provide a lead in developing feather biological isoscapes for migratory waterfowl, enabling the creation of species-specific maps for individual birds that are related to ground water. Furthermore, by creating a global network of collection sites, it will provide the inputs needed to acquire foundation data for precipitation isoscapes in regions where GNIP does not have sufficient coverage. Another important function will be production of isotopic keratin reference standards for primary reference materials containing exchangeable hydrogen. The need for such materials is essential to enable  $\delta D$  analysis to become a reliable tool for accurately predicting the origin of wild birds.

Radiation inactivation is a technique that has enormous potential for producing vaccines against pathogens that have so far proved intractable in finding methods to protect against infection (please note our planned vaccine irradiation CRP for 2010). These problematic diseases include parasitic infections like Fasciola trypanosomosis and Schistosoma that affect livestock, but also cause zoonotic infections in humans, and viral infections like Rift Valley fever and FMD. Current vaccines, if available, are not very effective and attempts to create engineered vaccines have proven genetically unsuccessful. A key initial research issue will be to establish improvements in radiation technology for processing and attenuating pathogens and adopting standardized procedures for the preservation, handling and transport of immunogenic material. Investigations will centre on using attenuating doses of gamma irradiation that disrupt gene expression in the infective stages of parasites, parasitic helminths or mechanically transmitted trypanosomes (T. vivax, T. evansi) so that their migration and development in the skin is retarded, allowing the immune system to recognize key parasite molecules and to mount a protective response. Applications regarding irradiated bacterial and viral vaccines will follow.

There is considerable genetic diversity among sheep and goat breeds in Asia, but lack of a coherent breeding strategy has meant this resource is under-utilized. For instance, biodiversity might be expressed in ability to resist endemic disease, or harsh environments (please see our planned CRP for 2010). The IAEA has worked with MS in Asia to enable them to acquire information on genetic variation of livestock breeds and start the mapping of genes controlling desirable traits in order to integrate them into breeding programmes. A computer program for MS allows them to input data, provides access to laboratory protocols, standard operating procedures for gene analysis and tools for genome searches and a livestock molecular markers database. The DNA of Asian sheep and goats breeds has been sequenced with the participation of an IAEA Collaborating Centre in Brazil. DNA and phenotypic farming system information has been acquired from over 4000 sheep and goats of 89 breeds, and 40 breeds of goat and sheep have been genotyped for 15 microsatellite markers. The IAEA is also participating in the Sheep HapMap project of the International Sheep Genomics Consortium in Australia to acquire detailed information regarding the location of genes affecting important productive traits in the African Dorper sheep.

The evaluation of existing management practices and their improvement to enhance reproductive efficiency, especially in ruminants is been thoroughly supported with the application of various protocols and procedures involving the use of progesterone radioimmunoassays, sets of milk or blood samples collected at specific times and in-house computer applications (e.g. AIDA Asia, AIDA Africa, LIMA) for data recording, analysis and interpretation of results. By using this, age at first service and at first calving, intervals from calving to conception and calving intervals have been reduced by several months in several tropical countries where Zebu cattle or crossbreeds are reared. Also, artificial insemination services has been improved by increasing the number of inseminated cows, reducing the number of services per conception, and avoiding the use of bulls with impaired semen quality.

Both past and future activities are described in detail in this newsletter and are also accessible at our website (http://www-naweb.iaea.org/nafa/aph/index.html); I thus need not mention them in this section. Please contact us if you have any further ideas, comments, concerns or questions. As discussed in previous newsletters, the Animal Production and Health Sub-programme will continue to move progressively forward and in pace with developments within the livestock field, to optimally serve our Member States. Please, contact us in case you would like to receive printed copies of the Symposium book of extended synopses for distribution in your institutes and universities. Concerning news from the Sub-programme, we want to welcome Linda Fuga and Barbara Rouchouze and wish them all the best in the Subprogramme.

Finally, I wish you all and your families a happy, healthy and safe New Year.

. thyou

Gerrit Viljoen, Head, Animal Production and Health Section

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Oie

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

# **Animal Production and Health Sub-programme**



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# **Forthcoming Events**

## **Consultants Meeting on Genetic Varia**tion on the Control of Resistance to Infectious Diseases

Technical Officer: Mario García Podestá

The meeting will be held from 8 to 10 February 2010 in Vienna, Austria.

The purpose of the meeting is to revise the work plan of the CRP on 'Genetic variation on the control of resistance to infectious diseases in small ruminants for improving animal productivity' with special emphasis on the candidate gene analysis or the genome wide approach methodologies, and to devise a suitable protocol for recording phenotypic data from sheep and goat breeds that are apparently resistant or non-resistant to helminth parasitism. More details are available under New CRP in this Newsletter.

## **Consultants Meeting on the Early and Rapid Diagnosis and Control of Footand-Mouth Disease**

Technical Officer: Gerrit Viljoen

The meeting will be held from 12 to 14 April 2010 in Melbourne, Australia.

Foot-and-mouth disease (FMD) is one of the most important livestock diseases known to man due to its high infection rate (ease of spread) and its effect on the limitation of livestock movement and trade. An outbreak of FMD will have a devastating effect on a country's food security with direct impact on national and international trade. The confirmatory diagnosis of FMD and its effective control through prophylactic, quarantine or slaughter-out procedures are therefore of paramount importance as it has financial and trade implications. Vaccination with inactivated FMD virus is undertaken to control FMD in endemic countries or countries at risk. Vaccines, whilst widely available but which should match (i.e. should be of homologous serotype and strain isolate) with virulent FMD viruses circulating in the region of vaccine use, are of variable quality, not from the homologous outbreak serotype/strain isolate, and are often stored under inadequate temperature conditions and therefore might be not as effective in the field as determined in animal experiments. Due to insufficient knowledge on vaccine strength and antigenic match (antigenic cartography) between vaccine strain and outbreak virus, it is often not possible to pinpoint the weakness of the vaccination strategy and to take action on this weakness.

Vaccine effectiveness can be determined by animal challenge, but this is both costly and difficult. In-vitro systems have been developed in different countries since the 1980's, but these are not standardized for international use. Many countries now produce FMD vaccines but often without consideration of their effectiveness. This consultant meeting will investigate early and rapid diagnostic and prophylactic methods and possibly provide internationally acceptable guidelines for procedures which test a vaccine's ability to induce the production of protective antibodies in cattle without the need for animal challenge experiments.

In many developing countries, vaccination will continue to be an essential component for the progressive control of FMD. Maximizing the effectiveness of current vaccines and supporting research to improve the effectiveness and quality of those and or new vaccines will be critical. Countries using locally produced vaccines need to assure trade partners that they are using quality assured vaccines in order to overcome the restrictive effects of endemic FMD. The provision of internationally accepted guidelines for quality assurance and alternatives to the present need for animal challenge vaccine trials would be a significant step forward. It is likely that control and eventual eradication in endemic areas with a low level resource base (much of Africa, parts of Asia and Latin America) will require the use of quality assured vaccine preparations, correct vaccine formulations (i.e. homologous strain or isolate vaccine to protect against outbreak, new generation vaccines with a broader protection base (i.e. cross protection between different strains and isolates) or alternative formulations of existing vaccines.

Specific Consultation Objectives will be:

- a) Discuss methods and protocols for measuring the potency of FMD vaccines using in vitro methods.
- b) Discuss and propose guidelines for optimum population vaccination intervals based on in vitro measurements of potency and duration of the antibody response to structural proteins, after vaccination of cattle and small ruminants with commercially available FMD vaccines, including evaluation of reduced dose options such as intra-dermal administration of FMD vaccine;
- c) Discuss and evaluate protocols and guidelines for application and interpretation of vaccine matching methods (antigenic cartography) to identify the extent of expected cross-protection of type A or SAT viruses;
- d) Discuss and evaluate the most appropriate early and rapid diagnostic technologies.

## **Consultants Meeting on the Effect of Climatic Change on Animal Production and Health – Way Forward**

Technical Officer: Hermann Unger

The meeting will be held in May/June 2010 in Vienna, Austria.

Abnormalities in weather and climate not only affect agriculture through droughts and flooding but also through changing patterns of disease occurrence and distribution. The direct influence of climate changes on vector-borne diseases is obvious, but inherent factors specifying such changes are still widely unknown. Additionally, the extreme weather conditions also interfere with productivity and disease resistance in livestock. The consultants meeting will evaluate future trends of climatic change, its impact on livestock production in general and more specific on vector-borne diseases and on genetic traits supporting increased performance under harsh or changing climatic conditions. The meeting should result in guidelines on issues and/or activities the Joint FAO/IAEA division should focus on to alleviate constraints arising from global warming and climatic changes.

## Research Coordination Meeting on the Early and Rapid Diagnosis of Transboundary Animal Diseases: Phase 1 – Avian Influenza

Technical Officer: Gerrit Viljoen

The Final Research Coordinated Meeting on 'The Early and Rapid Diagnosis of Transboundary Animal Diseases: Phase I - Avian Influenza' Coordinated Research Project will be held from 10 to 14 May 2010, in Rome, Italy. A full report on the CRP can be found under Coordinated Research Projects in this Newsletter.

## Research Coordination Meeting on the Use of Irradiated Vaccines in the Control of Infectious Transboundary Diseases of Livestock

Technical Officer: Antony George Luckins

The meeting will be held in April/May 2010 in Vienna, Austria.

Research Agreement Holders will present an up-to-date briefing of current theory and practise of vaccine technology and outline the work that they are engaged in. Research Contract holders will present their intended work plans that will be discussed by all participants and critically reviewed by Agreement Holders and Technical Officers. Revised work plans will be formulated and programmes of work on the projects for the next two years approved.

## Final Research Coordination Meeting of the Regional TC Project RLA5049 on 'Integrated Control of Fascioliasis in Latin America (in support of national programmes)

Technical Officer: Gerrit Viljoen/Kathrin Schaten The final coordination meeting will be held in La Paz, Bolivia, from 22 to 25 February 2010.

During the meeting the results obtained in the Project from 2007-2009 will be presented through oral presen-

tations and a final report. Upon obtaining the results, a work plan for an extension or a new project will be developed and discussed. Eligible Latin American participants should be actively involved in the diagnosis and control of fascioliasis. The meeting will take place in Spanish and English.

Agricultural biotechnologies in developing countries: Options and opportunities in crops, forestry, livestock, fisheries and agro-industry to face the challenges of food insecurity and climate change (ABDC-10), Guadalajara, Mexico, 1-4 March 2010

This FAO international technical conference is coorganized by FAO and the Government of Mexico, and co-sponsored by the International Fund for Agricultural Development (IFAD). The Consultative Group on International Agricultural Research (CGIAR), the World Bank and the International Centre for Genetic Engineering and Biotechnology (ICGEB) are major partners in this initiative. Participation at the conference is by invitation only.

Impetus for the conference comes from the need for concrete steps to be taken to move beyond the 'business-as-usual' approach and to respond to the growing food insecurity in developing countries, particularly in light of climate change that will worsen the living conditions of farmers, fishers and forestdependent people who are already vulnerable and food insecure. The conference encompasses the crop, forestry, livestock, fishery and agro-industry sectors, as well as the entire range of agricultural biotechnologies currently available.

For more information visit: http://www.fao.org/biotech/abdc/conference-home/en/

# Past Events

# Training Course on Advanced Molecular Diagnosis and Characterization of Influenza A (H5N1) and (H1N1)

Technical Officers: Gerrit Viljoen and Adama Diallo The meeting was held from 22 September to 2 October 2009 at Seibersdorf, Austria.

It was co-funded by the European Commission (Conflutech project), the IAEA and the FAO. This two-week training course aimed at enhancing knowledge on both the newly Influenza virus H1N1, called 'swine influenza' and the highly pathogenic avian influenza H5N1 (advanced molecular diagnosis of viruses 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 influenza A viruses (such as H1N1 and H5N1). The ultimate goal is to contribute to the early detection and early reaction capabilities in Member States. Eighteen participants from East Europe, Central Asia and Africa participated attended the training course.

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

Technical Officer: E. Nicholas Odongo

The meeting took place in Vienna, from 12 to 14 October 2009.

The purpose of the meeting was to develop a Coordinated Research Project (CRP) to investigate the biology of the digestive system and nutrient requirements of livestock during compensatory growth and restricted periods of growth and how to take advantage of the compensatory growth to maximize output.



Left to right: C. Park, N. Odongo, D. Poppi, C. Prasad, D. Gerrard and C. Rehfeldt

Five experts in animal growth, ruminant nutrition, muscle biology and lactational biology from Agricultural Research Organizations and Universities in Australia, Germany, India and USA attended the meeting, along with IAEA staff to discuss the potential of using the compensatory growth phenomenon to augment animal productivity.

Compensatory growth or 'catch-up growth' is a period of increase weight gain following a period of nutritional deprivation and is a common feature amongst animals. During this period of compensatory growth, animals exhibit greater body weight gain, increased efficiency of energy utilization, reduced maintenance requirements because of depression of the basic metabolic rate, enhanced appetite and feed intake capacity, changes in endocrine status, and altered body tissue composition compared with animals fed conventionally. Studies have shown that an animal's ability to compensate for prior periods of restricted feeding or under-nutrition is affected by severity and duration of the under-nutrition period, the animal's stage of development, genotype and sex, level of feed intake during re-alimentation, duration of re-feeding, and composition of the diet during re-alimentation. Because of the dramatic fluctuations in the quantity and quality of feedstuffs available for feeding animals year-round, compensatory growth is often of considerable practical significance to grassland livestock production. Even so, however, the physiological reason for the 'catch-up growth' has not been fully elucidated. In particular, little is known regarding the factors involved in partitioning nutrients following periods of under-nutrition or restricted feeding. Moreover, the requirements for maximizing output at this period of exaggerated growth are unknown.

The experts recognized the advantages the Joint FAO/IAEA Division had in coordinating integrated technical and research programmes through its Coordinated Research Projects (CRP) programme that bring together research institutes in both developing and developed Member States to collaborate on the research topic of interest. The meeting recommended the IAEA should consider initiating a new CRP on 'Use of the *compensatory growth phenomenon to improve livestock* productivity in regions with fluctuating quantity and quality of available feed resources'. Such an initiative is strongly supported by the growing need to secure sufficient supply of balanced feed resources for sustainable development of the livestock sector from resources, which do not compete with human food e.g. the use of tree leaves, agro-industrial by-products, and aquatic sources to bridge the gap between supply and demand of feeds. This initiative would build on the expertise already developed through the IAEA supported projects. The overall objective of the CRP is to improve livestock productivity using the compensatory growth phenomenon in environments with fluctuating feed supply. The first phase of the CRP would be to understand how animals lose and gain weight during periods of dramatic shifts in nutrient supply. Once the biological phenomenon that controls 'compensatory growth' has been elucidated, the second phase would develop management strategies to exploit and harness this highly efficient growth process to improve livestock production.

# Consultants Meeting on the Socio-Economic Impact of Disease Prevention

Technical Officer: Kathrin Schaten

The meeting took place in Vienna, from 2 to 4 November 2009.

Five experts in epidemiology, animal health economics, impact assessment, participatory approaches and costeffectiveness from Research Organizations and Universities in Great Britain, Netherlands, Ethiopia, Benin and USA attended the meeting, along with IAEA staff to discuss and evaluate methods to measure the socioeconomic impact of a disease and its prevention, diagnosis and treatment; how to get necessary information and what to do for long-term sustainability. Another objective of the meeting was to find a way to measure the impact on the global community that organisations like ours or others have. Furthermore it was helpful to discuss the advantage of the integration of more stakeholders; determine the key issues that need addressing; know how different they are in rural areas, cities, developing countries. By answering some of these questions it will enable us to recommend to governments how they can make the right decisions and create effective policies.

The report created in this meeting recommends a general framework and series of steps to develop detailed standardized methodologies to assess socioeconomic impact of disease prevention. It is important to understand the socioeconomic impact of diseases and their prevention in order to set priorities for intervention. Resources are quite limited for control of animal diseases. It is essential that these limited resources are used in a manner that will have the largest impact on improving and maintaining the socioeconomic status of society. In order for decision makers to prioritize the use of resources, more information is required on the socioeconomic impact of diseases and on the costeffectiveness of various interventions.

Furthermore any project to control animal diseases should follow Step 1-characteristics of diseases, step 2detailing the framework for impact of animal diseases and step 3-assessment of importance, so that the socioeconomic impact of disease and prevention can be clearly determined. The meeting recommends the development of detailed, standardized, globally applicable methods for determining Step 1, Step 2 and Step 3. Step 1 classifies diseases according to their zoonotic effect, the level of occurrence, the production effects, trade effects and the species affected. Step 2 details the measure needed for the assessment and how to generate them. Step 3 considers the calculations necessary to assess the impact of a project. As these Steps are currently written, there seems to be substantial overlap among them. The methods that the consultants meeting recommends developing would start with a clear set of definitions of each step and the parameters and limits of each to carefully differentiate them. These standardized, globally applicable methods would detail data sources currently available and ways to collect new information and techniques for integration of information from various sources. Finally, the obtained data are put in relation to socio-economic indicators, e.g. profit of new control measures, poverty indices etc., to estimate the impact a project had.



Back row, left to right: K. Schaten, M. Thrusfield, A. Luckins, P. Adegbola, H. Hogeveen. Front row: J. Walsh and B. Abegaz.

# Task Force Meeting on sustainable utilization of information and communication technologies for human resource development; applications for Veterinary laboratories and services; RAF0026

Technical Officer: Hermann Unger

The meeting was held in 16 – 20 November 2009 in Bamako, Mali

Documentation is one of the prerequisites for ISO certification of veterinary laboratories. The existing data management tools for veterinary laboratories are not seen as suitable for the needs, specifically in developing countries. During the first RAF 5057 coordination meeting, the need for a laboratory information and management system (LIMS) were expressed and their development discussed. For this task force meeting experts and consultants were invited to develop the structure of such software and to advice on the content. It was concluded that the programme should follow the pathways of a specimen along the diagnostic procedures, and that around a core software for data management, specific modules for the different diagnostic

procedures are build to reflect the institutional structures of laboratories (pathology, bacteriology, serology) but allow flexibility in assigning a task. The connection to technical equipment like ELISA readers or RT-PCR machines should be included as well to allow a precise data transfer and quality assurance procedures to be automatically build in. The integration of epidemiology modules for the evaluation of surveillance data is recommended.

The underlying software will be open source and as such allowing programming of specific modules by individual labs to address their needs. To connect the field veterinary services better to the central veterinary laboratories a special module will be developed to allow the rapid information exchange on disease occurrence and outbreaks using GSM technology. Altogether this vet-LIMS should help to speed up the diagnosis of disease and reporting country wide, improve the documentation and quality assurance procedures and should result in a more effective disease control finally as well at the regional level.

# **Coordinated Research Projects**

# **ACTIVE CRP**

#### Peste des Petits Ruminants (PPR)

Technical Officer: Adama Diallo, Hermann Unger This CRP has been running for two years. The overall objective is to develop, validate and transfer to Member States sensitive, specific and rapid tests for the diagnosis of PPR to help them better manage and control this transboundary animal disease. The activity received from the different research contract holders indicate the widespread prevalence of PPR in the different countries. For this activity the competitive PPR ELISA based on the recombinant N-protein of the virus was tested in the different laboratories. Problems were faced with some of the kit components but at the moment the test is running fine and results of this evaluation are expected at the next RCM. The molecular diagnosis of PPR was addressed in a number of laboratories. Good result were reported using filter paper as the transport matrix of samples, specifically for nasal discharge and the PCR procedures are now established in most laboratories. Dr. Gang Li recently developed an isothermic loop-mediated amplification for PPR. This procedure is much more robust then the conventional PCR, does not depend on a PCR cycler and can be done without an RNA extraction before the amplification. At the same time the sensitivity of this procedure is similar or even better then conventional or nested PCR. In cooperation with the Veterinary University Vienna and APU this test will now be further developed into a kit format and should be ready for field testing in early 2010.

All the contracts were renewed recently and it is planned to organize a ring test in 2010 on the detection of PPRV by molecular techniques.

Finally, we are very sorry to announce that one of the agreement holders of this CRP, Professor Tom Barrett from the Institute for Animal Health in UK, passed away in September. Prof. Barrett has made important contributions improving the diagnosis of rinderpest and PPR by serological and molecular methods. We have lost not only a good colleague but a dear friend.

## The Early and Rapid Diagnosis of Transboundary Animal Diseases: Phase I - Avian Influenza

#### Technical Officer: Gerrit Viljoen

This Coordinated Research Project (CRP) focuses on the early and rapid diagnosis and control of avian influenza (as technological target) through the advantageous use of nuclear, nuclear associated and nuclear related technologies, in conjunction with nonnuclear technologies. In particular, the 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), and the use of isotopes (P32/33, S35 and S35Met) to label or trace virus nucleic acid or proteins during development and comparative phases of research, and for the evaluation or characterization of targeted genes.

The overall objective is to develop, evaluate and validate early and rapid detection technologies to provide Member States (MS) with the capacity to detect, monitor, contain and control transboundary animal diseases (TADs). The CRP is supporting 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 are pertinent to all other TADs since the technologies addressed in this CRP form part of an early response diagnostic capability platform. 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, nuclear associated and nuclear related technologies and the harmonization and dissemination 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 prophylactic measures (e.g. 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 its 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 current 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 that 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 weekold 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,

Outputs:

using either fluorescent or isotopic [P32, P33 or S35] markers). This molecular approach takes 1-2 days to complete. Furthermore, it is foreseen that this technology could be applied as early warning tools. This technological platform has been extended to also include the Influenza A H1N1 subtype virus (swine flu). 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. Developments reported at the consultant's meeting indicated that increased feasibility of testing technological platforms with potential to satisfy criteria of mobility and flexibility required for field as well as laboratory use are real possibilities.

Expected	Present Status
Protocols and SOPs for the differentiation of disease agents including avian influenza with defined limits for testing according to OIE guidelines.	Conventional and real-time PCR platforms were developed and are in the process of evaluation and validation. The two step platforms are more sensitive, but more contamination prone, than the one step platforms. All the Research Contract Holders (RCHs) demonstrated some level of proficiency in the technology. Eight RCHs participated in basic molecular diagnostic training courses, but will need a more advance course. A working manual is in preparation.
Agreement on specific systems to be examined and methods reported.	The CRP selected the best technological platforms (equipment and their applied chemistries). These platforms, for example the TACMAN and hybridization chemistries and micro-arrays are being evaluated by selected RCHs.
Harmonized SOPs that are easy to perform, mobile (compact), robust, rugged and where results can be read unambiguously by relatively untrained staff and that are compatible with international databases.	The molecular (PCR and LAMP) SOPs will be presented following the finalization of the validation process. Several RCHs are participating in a ring trial exercise.
Development and evaluation of machines, primers and reagents to allow development of laboratory based tests and validate the tests to OIE stage 1 and 2 by comparison with conventional (accepted tests) using both serum samples and those for direct antigen testing where possible.	The molecular diagnostic platforms that were selected by the RCHs (implementation of applied chemistries) are being further developed (optimized and fine-tuned) and the final products will be tested by RCHs with endemic avian influenza (or at risk to) in their respective countries.
Modification of laboratory based systems to allow for mobility and simplicity in the field.	The molecular diagnostic platforms that were selected by the RCHs (equipment) are being further developed and the final products will be tested by RCHs with endemic avian influenza (or at risk to) in their respective countries.
Validation of mobile tests to OIE stage 1 and 2. Field testing of mobile isothermal devises in a routine diagnostic environment and comparison of sample data to laboratory based testing.	This will start in the final year of the CRP.

Overall Assessment of Progress towards Achieving Objective:

Progress towards achieving the objectives is satisfactory and the continuation of the CRP is recommended. This will allow for the standardization and harmonization of procedures and the feasibility testing of equipment with potential.

- Nuclear, nuclear associated and nuclear related molecular diagnostic assays (several PCR and PCR sequencing platforms) were developed, evaluated and are in the process of validation.
- Molecular procedures were harmonized and are available as SOPs. They are being ring-tested in 8 RCH laboratories. They were successfully implemented in endemic countries such as China, Indonesia, Nigeria and Egypt.
- RCHs were trained in molecular technologies and quality assurance management. Guidelines and a manual are available.
- Epidemiological and surveillance (including sampling frame) strategies were developed and are implemented by all RCHs.
- The training of RCHs was facilitated through other funds to help achieve the objectives of the CRP.
- The most appropriate technologies (equipment and their respective chemistries) were identified and pursued with commercial companies with RCH participation and buy-in. It is expected that at least two platforms (Late PCR and LAMP) will be tested in the Agreement reference laboratories 2009/2010.

## **Control of Contagious Bovine Pleuro-Pneumonia (CBPP)**

Technical Officer: Hermann Unger

This CRP is now entering its last year. The validation of the CBPP c-ELISA is now completed and publications on the findings are under way. Currently a ring test for all CRP members and as well for RAF5057 MS is organized by F. Thiaucourt, CIRAD. Due to logistical problems, not all tests could be sent out in time, but it's hoped that the flowback of information will help to assess the capacity of the participating labs to diagnose CBPP by serology.

The re-development of the LPPQ ELISA was not met with the success necessary to promote a commercial production due to a relatively high background demanding a serum dilution of 1/400, which was not practical. The original test performed very well on a serum dilution of 1/10. The molecular diagnosis of CBPP is now well addressed and a number of laboratories are performing PCR and q-PCR. Recently an isothermic CBPP Loop-mediated Amplification was developed by the Veterinary University Vienna together with APU. First experiments in Mali ascertained the performance of the system but showed some intrinsic problems which now will be addressed by evaluating the best location for the outer primers and the sample preparation method. The final RCM for this CRP is planned to be in Zanzibar in the last week of September 2010 (27.9. - 1.10.). All research contract holders are invited to come up with their research results in a format which will allow the publication in a book format tentatively titled 'CBPP control, the way forward'.

# **NEW CRP**

# The Use of Irradiated Vaccines in the Control of Infectious Transboundary Diseases of Livestock

#### Technical Officer: Antony George Luckins

The livestock sector is an important source of income for small holder farmers in the developing world. The growing demand for livestock products driven by population growth will provide the rural poor additional opportunities to increase livelihoods. Paramount to meeting this demand will be the need for governments to improve animal health, particularly where it relates to control of infectious diseases since they are a major constraint on livestock productivity and there is an urgent need to tackle the problem in order to ensure food security. Although diseases caused by viruses and bacteria are of major concern, often causing serious epidemics and compromising international trade, parasitic diseases caused by helminths such as Fasciola and Schistosoma or protozoa like Trypanosoma and Theileria exert a persistent, debilitating effect on livestock productivity throughout Africa, Asia and South America. With one or two exceptions their control relies on chemotherapy, however, this approach has a number of disadvantages. Firstly, even though animals are cleared of infection rapidly after treatment, it often fails to prevent re-infection thereby requiring frequent administration of the drug. Secondly, parasites are able to adapt genetically to the action of the drugs, resulting in the development of drug resistance – a common cause of overuse or inaccurate administration. This latter problem is compounded by the widespread use of fake products in many MS. Also, long term treatment brings with it the accumulation of drug residues in meat and milk, a situation that could compromise export of livestock products to the developed economies.

While this is a strong reason to develop vaccines against parasitic diseases, it will be necessary to apply innovative techniques to achieve this aim since in the past there has been only limited success in producing effective vaccines. Although a few attenuated, live vaccines are available they have a limited shelf life and it can be difficult to select appropriate, genetically attenuated organisms. Recombinant vaccines have also failed to live up to their promise, and there are a number of reasons for this. Parasites are complex organisms, comprising thousands of proteins, and identifying a single protective antigen is difficult, if not impossible since the immune response is multifaceted, requiring activation of several different immune pathways and it is synergy between different antigens that enables this to occur.

The way in which this can be achieved is through the use of gamma radiation attenuated organisms where there is strong evidence that both cellular and humoral immune responses are activated, simulating the response that occurs when live organisms are introduced into the host. Irradiation of whole organisms obviates the need to identify specific antigenic components required for subunit vaccines, or the time and resources to create genetically attenuated organisms. Moreover, although radiation attenuated organisms are metabolically active and follow a similar migration route to nonirradiated organisms in the host they fail to develop into a mature infection. Gamma irradiation is also practicable for use with those bacteria or viruses where there are currently no effective vaccines available - irradiation will more efficiently preserve the antigenic and adjuvant structures destroyed by conventional chemical or heat treatment. Effective storage and delivery of vaccines is an essential part of a strategy for control of animal diseases and developments in cryobiology enabling lyophilization of whole cells will make it possible freeze-dry vaccines, even those prepared from helminths or protozoa. Radiation attenuation would also expedite rapid emergency vaccine production during epidemic outbreaks of microbial diseases and freeze-drying would enable such vaccines to be stored and transported without need for a cold chain, thereby benefiting resource-poor MS.

The CRP will: -

- a) Develop techniques for irradiation attenuation of, for example, *Trypanosoma, Theileria, Fasciola, Schistosoma* and RVF and FMD virus
- b) Use nuclear techniques to assess metabolic activity of irradiated organisms and follow their migration and establishment in the host from site of injection
- c) Test irradiated vaccines in experimental animals to determine protective dose and monitor level and duration of immunity
- d) Develop techniques for the lyophilization of irradiated vaccines and SOPs for their vitrification and lyoprotection and determine conditions for storage to preserve integrity of vaccine
- e) Develop flow-through irradiation techniques to enable fast preparation of vaccines

#### **Control of Foot-and-Mouth Disease**

#### Technical Officer: Gerrit Viljoen

Foot-and-mouth disease (FMD) is one of the most important livestock diseases known to man due to its high infection rate (ease of spread) and its effect on the limitation of livestock movement and trade. An outbreak of FMD will have a devastating effect on a country's food security with direct impact on national and international trade. The confirmatory diagnosis of FMD and its effective control through prophylactic, quarantine or slaughter-out procedures are therefore of paramount importance as it have financial and trade implications. Vaccination with inactivated FMD virus is undertaken to control FMD in endemic countries or countries at risk. Vaccines, whilst widely available but which should match (i.e. should be of homologous serotype and strain isolate) with virulent FMD viruses circulating in the region of vaccine use, are of variable quality, not from the homologous outbreak serotype/strain isolate, and are often stored under inadequate temperature conditions and therefore might be not as effective in the field as determined in animal experiments. Due to insufficient knowledge on vaccine strength and antigenic match (antigenic cartography) between vaccine strain and outbreak virus, it is often not possible to pinpoint the weakness of the vaccination strategy and to take action on this weakness.

Vaccine effectiveness can be determined by animal challenge, but this is both costly and difficult. *In-vitro* systems have been developed in different countries since the 1980's, but these are not standardized for international use. Many countries now produce FMD vaccines but often without effective consideration of their effectiveness. This CRP will investigate methods and possibly provide internationally acceptable guide-lines for procedures which test a vaccine's ability to induce the production of protective antibodies in cattle without the need for animal challenge experiments.

In many developing countries, vaccination will continue to be an essential component for the progressive control of FMD. Maximising the effectiveness of current vaccines and supporting research to improve the effectiveness and quality of those and or new vaccines will be critical. Countries using locally produced vaccines need to assure trade partners that they are using quality assured vaccines in order to overcome the restrictive effects of endemic FMD. The provision of internationally accepted guidelines for quality assurance and alternatives to the present need for animal challenge vaccine trials would be a significant step forward. It is likely that control and eventual eradication in endemic areas with a low level resource base (much of Africa, parts of Asia and Latin America) will require the use of quality assured vaccine preparations, correct vaccine formulations (i.e. homologous strain or isolate vaccine to protect against outbreak, new generation vaccines with a broader protection base (i.e. cross protection between different strains and isolates) or alternative formulations of existing vaccines.

The CRP will:

- a) Establish methods and develop internationally agreed protocols for measuring the potency of FMD vaccines using in vitro methods;
- b) Establish guidelines for optimum population vaccination intervals based on in vitro measurements of potency and duration of the antibody response to structural proteins, after vaccination of cattle and small ruminants with commercially available FMD vaccines, including evaluation of reduced dose options such as intradermal administration of FMD vaccine;

- c) Establish protocols and guidelines for application and interpretation of vaccine matching methods (antigenic cartography) to identify the extent of expected cross-protection of type A or SAT viruses; and,
- d) Provide further global co-ordination of current research into FMD vaccines for use in endemic settings.

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

#### Technical Officer: Mario García Podestá

Farmers in developing countries, due to the pressure for higher animal output and to the 'advantages' of small number of highly specialized breeds from the developed world have been replacing or crossbreeding their local breeds with exotic animals for many years. The genetic improvement has been quite successful in many places; however, neglecting or upgrading indigenous animals with exotic breeds is deteriorating genetic diversity.

Much of the genetic biodiversity controls advantageous traits influencing adaptability to harsh environments, productivity, or disease resistance. However, these indigenous animals are underutilized in conventional breeding programmes, due to a lack of knowledge and failure to identify breeds and animals carrying the most advantageous traits.

There is well-documented evidence for within and between breed genetic variation in resistance to infectious diseases, namely gastrointestinal nematode infections, diseases due to mycotoxins, bacterial diseases including foot root and mastitis, ectoparasites such as flies and lice, and scrapie, the small ruminant transmissible spongiform encephalopathy. Due to this withinbreed variation, in many cases disease resistance is a heritable trait. This offers the opportunity to select animals for enhanced resistance to the disease. The feasibility of this approach 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 indigenous breeds with some degree of enhanced resistance as compared to exotic ones reared in the same environment, especially for gastrointestinal nematode infections. Therefore, the present CRP is aiming, through genomic studies using radiolabeled nucleotides in DNA hybridization, DNA characterization, and hybrid mapping procedures for identifying molecular markers of economic interest which will open possibilities in the future to select and breed animals for enhanced resistance to diseases.

The specific objectives of the CRP are:

a) To develop capacity in developing countries in the use of molecular and related technologies and cre-

ate opportunities for international research collaboration;

- b) To establish or improve programmes for animal identification and data recording for small ruminants in developing countries, allowing for the monitoring of production, reproduction and health traits and generating populations suitable for molecular genetic studies;
- c) To collect phenotypic data and DNA samples from goat and sheep breeds or populations within-breeds with history of infectious disease resistance;
- d) To develop expertise on the use and development of bioinformatic tools for the analysis of large datasets if genomic data related to parasite resistance in various breeds;
- e) To provide valid data for the identification of genetic markers associated to infectious disease resistance and to initiate the development of tools for molecular diagnostics and assisted breeding; and,
- f) To contribute on the development and use of nuclear technology for genomic research in small ruminants, including radiation hybrid map, Southern Blot with radioactive  $[\alpha^{-32}P]ATP$  labeling in genetic marker analysis, and PCR-RFLP.

Up to 14 research contracts will be awarded to Member States submitting appropriate research proposals. Institutions willing to participate in the CRP must be engaged in programmes of national importance in genetics and breeding of livestock, have access to basic laboratory facilities for studies on molecular genetics, have local support and facilities for phenotypic data collection, and be recipients of collateral financial support from national, bilateral, or international sources. In addition, four Research Agreements will be awarded to institutes that have expertise in specific areas of importance to the CRP.

The selection of participating institutes will consider the importance of the country and the target sheep and goat population in relation to the FAO Programme on Global Animal Genetic Resources (AnGR), and potential for future applications in generating and using populations with resistance to gastro-intestinal helminth parasites.

Applicants to research contracts are expected to work with a minimum of two breeds or populations withinbreeds of goats or sheep where one of them has recorded data or anecdotal history of parasite infection resistance; however, working with both species or with four groups within ones species would be desirable. Also, a minimum of 25 unrelated animals per breed or group will need to be sampled for SNP verification. Phenotypic data, including parasite disease resistance, for each breed or population, if not available, will need to be collected during the first two years of the project using funds others than the CRP. Scientists interested in this CRP are encouraged to send their applications by **31 January 2010**.

More detailed information of this CRP can be obtained in <u>http://www-naweb.iaea.org/nafa/aph/new-crp.html</u>

# 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 <a href="http://www-crp.iaea.org/html/forms.html">http://www-crp.iaea.org/html/forms.html</a>

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 <a href="http://www-tc.iaea.org/tcweb/default.asp">http://www-tc.iaea.org/tcweb/default.asp</a>

For further information contact Anna Lyn Dimailig (a.dimailig@iaea.org)

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

## Development of test using Southern Blot with radioactive $[\alpha^{-32}P]$ ATP labeling in genetic marker analysis

The southern blot assay using  $\left[\alpha^{-32}P\right]$  dATP-labeled probes was evaluated as a suitable method for the single nucleotide polymorphism (SNP) detection. Several candidate genes were selected for establishing this method. PCR, digestion with restriction enzymes (if available) and gel electrophoresis on selective DNA samples were carried out. After amplification by PCR and digestion by restrictions enzymes, the DNA corresponding to the selected gene is submitted to electrophoresis in agarose gel and transferred onto a filter. The DNA on the blot was hybridized with a using  $\left[\alpha^{-32}P\right]$ dATP-labeled probe to confirm or supplement the candidate gene results from the RLFP study. This method can be used for signal intensification if the PCR-RLFP bands are too weak, for detection of specific restriction fragments in case of multicut, and for detection and differentiation of SNPs if restriction enzyme cleavage sites are not identified in the SNP position. In the last case only DNA probes which contain a corresponding SNP in the middle are able to generate the expected results. By using this assay sequencing can be supplemented or even replaced.

# Completion the analysis of the PPRV N protein domains involved in proteinprotein interactions in the virus particles

As part of the approach to develop a marker vaccine for the control of peste des petits ruminants (PPR), the nucleocapsid protein, N, of the virus has been targeted for the introduction of the negative and positive markers. This protein is the most abundant viral protein and also the protein against which most of antibodies produced in the host during infections are directed. However, N is playing a critical role in the multiplication of the virus by interacting with:

- 1) N itself to form the nucleocapsid which envelops and protects the genomic RNA,
- 2) The phosphoprotein (P) and the RNA polymerase (L protein) to form a complex responsible of the replication and encapsidation of the genomic RNA,
- 3) The matrix protein (M) during the formation of the virus particle. Indeed through this interaction, the ribonucleocapsid is pulled into the vesicle formed

by the M protein and the host cellular membrane during the maturation of the virus

So the introduction of the markers into N protein should be done in away not to affect seriously these interactions essential for the replication of the virus. For the past four years, studies were carried out in APU to identify the protein domains involved in these different interactions.

The N-N interactions were studied. The results that were obtained showed that the N-terminus spanning the amino acid residues 1 to 241 is critical for the N-N interaction and the formation of the nucleocapsid. However, the peptide mapping study has demonstrated the involvement of a peptide of the C-terminus in the final structure of the nucleocapsid.

For the interaction of N with P, the C-terminal region seems to be main involved area but a peptide in the Nterminus of N seems to be the most important binding site. However, a single deletion in each of these domains doesn't affect the binding of P to N.

M protein interacts with N by binding to 5 peptides corresponding to 4 discontinuous regions. Those peptides, 4 in the central region of N and one in the C-terminus, are very well conserved between viruses of the morbillivirus group.

Based on all the above studies, a region has been identified as potential site for the negative marker in the PPR vaccine. 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 analyse 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, long-range 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 Aimone-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

#### **Fellows and Interns at APU**

With a fellowship grant from the Rothamsted International (UK), **Ms. Mechtilda Byela Byamungu** joined APU in last June for 8 months to develop a Loop mediated isothermal Amplification (LAMP) test for the detection of trypanosma nucleic acid.

**Ms Kimberley A.F. Schiller**, student at the Royal Veterinary College in London, was intern in APU from June 22 to August 6 2009. She participated in the molecular epidemiology of capripox viruses by sequencing and analysing the late transcription factor 4 gene (VLTF4 gene) of capripox virus strains of different geographical origins gene.

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).

**Mr Esayas Gelaye Leykun**, with a fellowship support from IAEA Technical Cooperation Department, was a fellow in APU from September 1 to November 30. He was trained on the monoclonal antibody production technology: fusion of spleen cells with myoloma, screening and selection of hybridomas, isotyping monoclonal antibodies and mapping their epitopes on the target protein.

# IAEA Collaborating Centre on Animal Genomics and Bioinformatics

The IAEA Collaborating Centre is composed by laboratories from three world class research and teaching Brazilian institutions [Animal Biochemistry and Molecular Biology Laboratory (LBBMA), São Paulo State University, UNESP, Araçatuba; Laboratory of Computational and Systems Biology (LCSB), Instituto Oswaldo Cruz - FIOCRUZ (Oswaldo Cruz Foundation). Janeiro: Rio de and Animal Biotechnology Laboratory (ABL), Animal Sciences Department, Escola Superior de Agricultura Luiz de Queiroz, University of São Paulo, Piracicaba. The Liaison Officer or the Centre is Dr. Jose Fernando Garcia (UNESP).

- LBBMA is part of UNESP, one the three public universities funded by the State of São Paulo, and one of the largest institutions of higher education in Latin America. The relatively new Veterinary Medicine School is located in the Araçatuba campus and has a well equipped research infrastructure, especially for animal production and health. LBMMA focus its research activity on animal physiology, biochemistry, and molecular genetics with emphasis on important phenotypic characteristics in cattle such as early puberty, growth rate, milk production, and disease resistance. The main techniques developed by LBBMA are DNA cloning and sequencing, microsatellite analysis, and high throughput gene expression analysis.
- The LCSB is one of seventy-one laboratories of the Oswaldo Cruz Institute. Activities in the laboratory focused to molecular characterization. are functional and comparative genomics of trypanosomatids using bioinformatics tools. LCSB has created an international network discussion list (tryplink-1), а genomic database called ProtozoaDB (http://protozoadb.biowebdb.org), organized international Internet conferences on trypanosomatids, carried out a scientific journal (Kinetoplastid Biology and Disease) and organized meetings, training courses and individual training to Latin America and African scientists.
- The ABL is part of the Animal Sciences Department, University of Sao Paulo (USP). This university is the largest institution dedicated to higher education and research in Brazil, and the third in size in Latin America. ABL is also part of the Organization of Nucleotide Sequence and Analysis (ONSA) of the São Paulo State Research Foundation (FAPESP), that is involved in the sequencing of the complete genome of the first plan

pathogen (Xilella fastidiosa) and other important agricultural organisms (other strains of Xilella, Xanthomonas), plants (sugar cane, coffee, eucalyptus) and livestock (chicken and cattle). The laboratory is currently involved in candidate gene research, quantitative trait loci mapping, transcriptomics, and gene expression projects in ruminants (cattle and sheep) and chicken.

The IAEA and the IAEA Collaborating Centre are working closely and in consultation with each other to increase capacity of NARS and Member States to use nuclear and nuclear related gene-based technologies in conjunction with conventional technologies to improve livestock productivity, and to enhance national and regional research and analytical capabilities in order to promote self-reliance and to accelerate national development. The programme for 2009 – 2012 includes:

#### Information collection and dissemination:

 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 (Animal Production and Health Section, Animal Production Unit at Seibersdorf Laboratories, and the Technical Cooperation Department).

# Development, application, and evaluation of new technologies:

- Characterization of relevant indigenous and adapted livestock breeds, primarily cattle, sheep, and goats from tropical regions to establish a reference platform for molecular genetic research.
- Generation of phenotypic and genotypic DNA data banks to facilitate the evaluation of genetic diversity and the validation of markers for future livestock breeding strategies using molecular genetic information.
- Development and characterization of molecular tools for livestock genetic analysis.
- Application of molecular diagnostic tools and molecular markers for marker assisted selection for the improvement of animal production in the tropics.

#### Assistance to the IAEA's training programme:

- The Centre may host individual fellowships for scientists of IAEA member States in the field of animal genetics, both for short term duration as well as for PhD degrees.
- The Centre staff may provide on-site training through expert assignments to national and regional TC projects and NAFA training courses.

# **Technical Cooperation Projects**

TC Project	Description	ТО
BEN/5/003	Veterinary Drug Residue Monitoring Programme <b>Objective</b> : To develop a capacity for veterinary drug residue monitoring in livestock products.	Unger Diallo
BEN/5/006	Improving Animal Health and Productivity <b>Objective</b> : To strengthen, diagnose, and control African swine fever, and increase animal productivity.	Unger Diallo
BKF/5/006	Establishment of Feeding Tables for Feedstuffs that are Locally Available to Stockholders in Burkina Faso <b>Objective</b> : 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 <b>Objective</b> : 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.	Garcia Podesta Unger
BOL/5/019	Implementing Molecular Techniques to Upgrade the Diagnostic Facilities of National Animal Health Programmes <b>Objective</b> : 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.	Luckins Schaten
BOT/5/005	Improving Diagnosis of Animal Diseases <b>Objective</b> : 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 <b>Objective</b> : 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) <b>Objective</b> : 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 <b>Objective</b> : To strengthen the diagnostic capacity of the Central Veterinarian Labora- tory (LACAVET) to monitor and control major animal diseases.	Unger
CAF/5/004	<ul><li>Improving Livestock Production Through Disease Control and Artificial Insemination</li><li><b>Objective</b>: To improve animal production in the Central African Republic through livestock disease control and improved breeding by use of artificial insemination.</li></ul>	Unger Garcia Podesta

TC Project	Description	то
CMR/5/015	Use of Nuclear Techniques for Improving Ruminant Productivity & Disease Control <b>Objective</b> : Develop capability for improved breeding by disease control and artificial insemination.	Garcia Podesta Unger
CMR/5/017	Improving Animal Productivity and Health <b>Objective</b> : To strengthen capacity and outreach regarding artificial insemination in ruminants, and to control livestock diseases impeding reproduction and productivity.	Unger Garcia Podesta
ERI/5/005	Zoonotic (diseases that can be transmitted from animals to humans) Disease Control and Analysis of Veterinary Residues in Foods <b>Objective</b> : 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.	Cannavan Unger Patel
ERI/5/006	Controlling Major Epizootic Diseases and Other Mycoplasma Infections of Livestock <b>Objective</b> : To improve the control of transboundary animal diseases, and continue the eradication of tuberculosis and brucellosis.	Unger Luckins
ETH/5/012	Integrating Sterile Insect Techniques for Tsetse Eradication <b>Objective</b> : To eradicate the tsetse fly from the southern Rift Valley, thereby creating an environment conducive to livestock development and improved agricultural pro- duction.	Feldman Parker Viljoen
ETH/5/014	Monitoring and Control of Major Animal Diseases <b>Objective</b> : 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 <b>Objective</b> : To aid identification and control of livestock diseases.	Luckins Unger
HON/5/004	Improving the Nutrition and Health Conditions of Livestock in Order to Increase Productivity and Reproductivity (Phase II) <b>Objective</b> : 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) <b>Objective:</b> To strengthen and improve livestock production in Honduras.	Garcia Podesta Odongo Viljoen
INS/5/034	Development of Environmentally Sound Livestock and Agricultural Production <b>Objective</b> : To improve livestock productivity without adversely affecting the envi- ronment through improved feed supplementation strategies, managing nutrient waste on farms and reducing methane emissions.	Odongo
IVC/5/030	Assessing the Genetic Profile for Improved Livestock Production <b>Objective</b> : To assess the genetic profile of livestock for the effective revival of stockbreeding in Côte d'Ivoire.	Garcia Podesta Unger
KEN/5/027	Assessment of Local Feed Resources for Enhancing Fertility and Productivity of Smallholder Dairy Cattle <b>Objective</b> : To assess the potential of local feed resources for enhancing the fertility and productivity of smallholder dairy cattle in the Nakuru District of Kenya.	Odongo Garcia Podesta
KEN/5/028	Applying Nuclear Based Techniques to Control Animal diseases <b>Objective</b> : 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.	Unger
MAG/5/016	Applying Nuclear Techniques to Optimize Animal Production <b>Objective:</b> To increase animal production through the improvement of animal health and control reproduction in the Amoron'i Mania region.	Garcia Podesta Odongo Luckins

TC Projec	t Description	ТО
MAU/5/002	<ul> <li><sup>2</sup> Improving the National Capacity in Diagnostics for Animal Diseases (Infection and Parasitic Diseases)</li> <li><b>Objective</b>: 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.</li> </ul>	Luckins Schaten
MAU/5/00	<sup>3</sup> Improving the National Capacity in Diagnostics for Animal Diseases (Infection and Parasitic Diseases) <b>Objective</b> : 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/5/023	<ul> <li>Improving National Capabilities for Characterization of Serotypes of Major Animal Diseases Using Molecular Biology Techniques</li> <li><b>Objective</b>: 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.</li> </ul>	Unger Viljoen Schaten
MON/5/01:	<ul> <li><sup>3</sup> Diagnosis and Surveillance of Transboundary Animal Diseases and Production of Diagnostic Reagents</li> <li><b>Objective</b>: To obtain international recognition of freedom from several transboundary 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.</li> </ul>	Luckins Viljoen
MON/5/01	6 Improving Productivity of Cattle, Camels and Yaks Through Better Nutrition and Reproductive Management <b>Objective</b> : 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/5/01	7 Supporting the Sustainable Production and Supply of Vaccines and Diagnostic Kits for Transboundary Animal Diseases <b>Objective</b> : To produce vaccines and diagnostic kits for transboundary animal dis- eases.	Viljoen Luckins
MOR/5/030	<ul> <li>Improving Sheep and Goat Production in Morocco through Genomic and Reproductive Physiology Characterization with the Help of Radio-immunoassay and Molecular Techniques (Not yet funded)</li> <li><b>Objective</b>: Increase sheep and goats for consumption and producers' revenue while preserving natural resources.</li> </ul>	Garcia Podesta Malek
MOZ/5/002	<ul> <li>Promoting sustainable Animal Health, Reproduction and Productivity Through the Use of Nuclear and Related Techniques</li> <li><b>Objective</b>: To obtain sustainable improvement in animal reproduction and breeding and animal health through the use of nuclear and nuclear related technologies.</li> </ul>	Viljoen
MYA/5/01	<sup>3</sup> Integrated Approach for Enhancing Cattle Productivity <b>Objective</b> : To improve smallholder dairy cattle production in Yangon and Mandalay regions.	Garcia Podesta Odongo
MYA/5/01	5 Strengthening the National Capacity for the Production of Veterinary Vaccines <b>Objective</b> : To enhance the national capacity for quality vaccine production to support efforts to control infectious diseases in livestock production, particularly FMD.	Unger Diallo

TC Project	Description	ТО
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 <b>Objective</b> : 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 Diallo Unger
NER/5/013	An Integrated Approach for Improvement of Livestock Productivity <b>Objective</b> : To increase the productivity of livestock through implementation of an integrated programme dealing with nutrition and reproduction.	Odongo Garcia Podesta Diallo
PER/5/029	Genomics of the Alpaca: Identification of Expressed Genes and Genetic Markers Associated with Productivity and Embryonic Mortality <b>Objective</b> : 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 Technologies (AFRA III-4) <b>Objective:</b> 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
RAF/5/055	Support to African Union's Regional Programmes for Control and Eradication of Major Epizootics <b>Objective</b> : 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.	Viljoen Lelenta
RAF/5/057	Strengthening Capacities for the Diagnosis and Control of Transboundary Animal Diseases in Africa (AFRA) <b>Objective</b> : 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.	Unger Diallo
RER/5/015	Supporting Early Warning and Surveillance of Avian Influenza Infection in Wild and Domestic Birds and Assessing Genetic Markers for Bird Resistance <b>Objective</b> : To establish early bird flu diagnosis and assessment of genetic markers for AI resistance with nuclear molecular methods in the region of Bosnia and Herze- govina, Bulgaria, Croatia, Macedonia, Montenegro, Serbia, Turkey, Uzbekistan, Kyrgyzstan and The Russian Federation.	Viljoen Diallo
RLA/5/049	Integrated Control of Fascioliasis in Latin America (in support of National Pro- grammes	Viljoen Schaten

TC Project	Description	ТО
SIL/5/006	Improving the Productivity of N'dama Cattle <b>Objective</b> : 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 dis- eases.	Garcia Podesta Odongo Viljoen
SIL/5/010	Improving the Productivity of Ndama Cattle In Sierra Leone <b>Objective</b> : To strengthen the diagnostic capacity to monitor and control animal diseases affecting cattle, (ii) to apply feeding strategies and supplementation pack- ages, and (iii) to produce hydrids with greater potential for increased growth rate and milk yields.	Garcia Podesta Odongo Viljoen
SIL/5/011	Controlling Economically Important Livestock Diseases <b>Objective</b> : 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 <b>Objective</b> : 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.	Odongo
SRL/5/042	Applying Molecular Diagnostics to Zoonotic Diseases <b>Objective</b> : 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 <b>Objective</b> : 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 <b>Objective</b> : 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 <b>Objective</b> : 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 <b>Objective</b> : 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.	Luckins
UGA/5/028	Improving the Capacity for Diagnostic of Animal Diseases <b>Objective</b> : 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) <b>Objective</b> : 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.	Unger Luckins

TC Project	Description	ТО
URT/5/025	Support for the Delivery of Artificial Insemination services <b>Objective</b> : 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 <b>Objective</b> : 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
ZAI/5/015	Upgrading Laboratory Services for Diagnosis of Animal Diseases <b>Objective</b> : 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 <b>Objective</b> : 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- able resources.	Garcia Podesta Odongo

# **Publications**

## Genome-Wide Survey of SNP Variation Uncovers the Genetic Structure of Cattle Breeds

The Bovine HapMap Consortium

The imprints of domestication and breed development on the genomes of livestock likely differ from those of companion animals. A deep draft sequence assembly of shotgun reads from a single Hereford female and comparative sequences sampled from six additional breeds were used to develop probes to interrogate 37,470 single-nucleotide polymorphisms (SNPs) in 497 cattle from 19 geographically and biologically diverse breeds. These data show 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 (Science (2009), 324: 528-532).

## Capripoxvirus G-protein-coupled chemokine receptor: a host-range gene suitable for virus animal origin discrimination

C. Le Goff, C.E. Lamien, E.Fakhfakh, A. Chadeyras, E. Aba-Adulugba, G. Libeau, E. Tuppurainen, D.B. Wallace, T. Adam, R.Silber, V. Gulyaz, H. Madani, P. Caufour, S. Hammami, A.Diallo and E. Albina

The genus Capripoxvirus within the family Poxviridae comprises three closely related viruses, namely goat pox, sheep pox and lumpy skin disease viruses. This nomenclature is based on the animal species from which the virus was first isolated, respectively, goat, sheep and cattle. Since capripoxviruses are serologically identical, their specific identification relies exclusively on the use of molecular tools. We describe here the suitability of the G-protein-coupled chemokine receptor (GPCR) gene for use in host-range grouping of capripoxviruses. The analysis of 58 capripoxviruses showed three tight genetic clusters consisting of goat pox, sheep pox and lumpy skin disease viruses. However, a few discrepancies exist with the classical virushost origin nomenclature: a virus isolated from sheep is grouped in the goat poxvirus clade and vice versa. Intra-group diversity was further observed for the goat pox and lumpy skin disease virus isolates. Despite the presence of nine vaccine strains, no genetic determinants of virulence were identified on the GPCR gene.

For sheep poxviruses, the addition or deletion of 21 nucleic acids (7 aa) was consistently observed in the 59 terminal part of the gene. Specific signatures for each cluster were also identified. Prediction of the capripoxvirus GPCR topology, and its comparison with other known mammalian GPCRs and viral homologues, revealed not only a classical GPCR profile in the last three-quarters of the protein but also unique features such as a longer N-terminal end with a proximal hydrophobic a-helix and a shorter serine-rich C-tail (*Journal of General Virology (2009), 90: 1967–1977*).

# International network for capacity building for the control of emerging viral vector-borne zoonotic diseases: ARBO-ZOONET

J. Ahmed, M. Bouloy, O. Ergonul, A.R. Fooks, J. Paweska, V. Chevalier, C. Drosten, R. Moormann, N. Tordo, Z. Vatansever, P. Calistri, A. Estrada-Peña, A. Mirazimi, H. Unger, H. Yin and U. Seitzer

Arboviruses are arthropod-borne viruses, which include West Nile fever virus (WNFV), a mosquito-borne virus, Rift Valley fever virus (RVFV), a mosquitoborne virus, and Crimean-Congo haemorrhagic fever virus (CCHFV), a tick-borne virus. These arthropodborne viruses can cause disease in different domestic and wild animals and in humans, posing a threat to public health because of their epidemic and zoonotic potential. In recent decades, the geographical distribution of these diseases has expanded. Outbreaks of WNF have already occurred in Europe, especially in the Mediterranean basin. Moreover, CCHF is endemic in many European countries and serious outbreaks have occurred, particularly in the Balkans, Turkey and Southern Federal Districts of the Russian Federation. In 2000, RVF was reported for the first time outside the African continent, with cases being confirmed in Saudi Arabia and Yemen. This spread was probably caused by ruminant trade and highlights that there is a threat of expansion of the virus into other parts of Asia and Europe. In the light of global warming and globalisation of trade and travel, public interest in emerging zoonotic diseases has increased. This is especially evident regarding the geographical spread of vectorborne diseases. A multi-disciplinary approach is now imperative, and groups need to collaborate in an integrated manner that includes vector control, vaccination programmes, improved therapy strategies, diagnostic tools and surveillance, public awareness, capacity building and improvement of infrastructure in endemic regions (Eurosurveillance (2009), 14: Issue 12).

## Feeding saponin-containing Yucca schidigera and Quillaja saponaria to decrease enteric methane production in dairy cows

L. Holtshausen, A.V. Chaves, K.A. Beauchemin, S.M. McGinn, T.A. McAllister, N.E. Odongo, P.R. Cheeke and C. Benchaar C.

An experiment was conducted in vitro to determine whether the addition of saponin-containing Yucca schidigera or Ouillaia saponaria reduces methane production without impairing ruminal fermentation or fiber digestion. A slightly lower dose of saponin was then fed to lactating dairy cows to evaluate effects on ruminal fermentation, methane production, total-tract nutrient digestibility, and milk production and composition. A 24-h batch culture in vitro incubation was conducted in a completely randomized design with a control (no additive, CON) and 3 doses of either saponin source [15, 30, and 45 g/kg of substrate dry matter (DM)] using buffered ruminal fluid from 3 dairy cows. The in vivo study was conducted as a crossover design with 2 groups of cows, 3 treatments, and three 28-d periods. Six ruminally cannulated cows were used in group 1 and 6 intact cows in group 2 (627 + -55 kg of body weight and 155 +/- 28 d in milk). The treatments were 1) early lactation total mixed ration, no additive (control; CON); 2) CON diet supplemented with whole-plant Y. schidigera powder at 10 g/kg of DM (YS); and 3) CON diet supplemented with wholeplant Q. saponaria powder at 10 g/kg of DM (QS). Methane production was measured in environmental chambers and with the sulfur hexafluoride (SF(6))tracer technique. In vitro, increasing levels of both saponin sources decreased methane concentration in the headspace and increased the proportion of propionate in the buffered rumen fluid. Concentration of ammonia-N, acetate proportion, and the acetate:propionate ratio in the buffered rumen fluid as well as 24-h digestible neutral detergent fiber were reduced compared with the CON treatment. Medium and high saponin levels decreased DM digestibility compared with the CON treatment. A lower feeding rate of both saponin sources (10 g/kg of DM) was used in vivo in an attempt to avoid potentially negative effects of higher saponin levels on feed digestibility. Feeding saponin did not affect milk production, total-tract nutrient digestibility, rumen fermentation, or methane production. However, DM intake was greater for cows fed YS and QS than for CON cows, with a tendency for greater DM intake for cows fed YS compared with those fed QS. Consequently, efficiency of milk production (kg of milk/kg of DM intake) was lower for cows fed saponin compared with controls. The results show that although saponin from Y. schidigera and Q. saponaria lowered methane production in vitro, the reduction was largely due to reduced ruminal fermentation and feed digestion. Feeding a lower dose of saponin to lactating dairy cows avoided potentially negative effects on ruminal fermentation and feed digestion, but methane production was not reduced. Lower efficiency of milk production of cows fed saponin, and potential reductions in feed digestion at high supplementation rates may make saponin supplements an unattractive option for lowering methane production *in vivo (Journal of Dairy Science (2009), 92: 2809-2821).* 

## NSs protein of rift valley fever virus induces the specific degradation of the double-stranded RNA-dependent protein kinase

M. Habjan, A. Pichlmair, R.M. Elliott, A.K. Överby, T.Glatter, M.Gstaiger, G. Superti-urga, H. Unger and F. Weber

Rift Valley fever virus (RVFV) continues to cause large outbreaks of acute febrile and often fatal illness among humans and domesticated animals in Africa, Saudi Arabia, and Yemen. The high pathogenicity of this bunyavirus is mainly due to the viral protein NSs, which was shown to prevent transcriptional induction of the antivirally active type I interferons (alpha/beta interferon [IFN- $\alpha/\beta$ ]). Viruses lacking the NSs gene induce synthesis of IFNs and are therefore attenuated, whereas the noninducing wild-type RVFV strains can only be inhibited by pretreatment with IFN. We demonstrate here in vitro and in vivo that a substantial part of the antiviral activity of IFN against RVFV is due to a double-stranded RNA-dependent protein kinase (PKR). PKR-mediated virus inhibition, however, was much more pronounced for the strain Clone 13 with NSs deleted than for the NSs-expressing strain ZH548. In vivo, Clone 13 was nonpathogenic for wild-type (wt) mice but could regain pathogenicity if mice lacked the PKR gene. ZH548, in contrast, killed both wt and PKR knockout mice indiscriminately. ZH548 was largely resistant to the antiviral properties of PKR because RVFV NSs triggered the specific degradation of PKR via the proteasome. The NSs proteins of the related but less virulent sandfly fever Sicilian virus and La Crosse virus, in contrast, had no such anti-PKR activity despite being efficient suppressors of IFN induction. Our data suggest that RVFV NSs has gained an additional anti-IFN function that may explain the extraordinary pathogenicity of this virus (Journal of Virology (2009), 83: 4365-4375).

#### Advances in viral disease diagnostic and molecular epidemiological technologies S. Belák, P. Thorén, N. LeBlanc and G. Viljoen

The early and rapid detection and characterization of specific nucleic acids of medico-veterinary pathogens have proven invaluable for diagnostic purposes. The integration of amplification and signal detection systems, including online real-time devices, have increased speed and sensitivity and greatly facilitated the quantification of target nucleic acids. They have also allowed for sequence characterization using melting or hybridization curves. The newer-generation molecular diagnostic technologies offer, hitherto, unparalleled detection and discrimination methodologies, which are vital for the positive detection and identification of pathogenic agents, as well as the effects of the pathogens on the production of antibodies. The development phase of the novel technologies entails a thorough understanding of accurate diagnosis and discrimination of present and emerging diseases. The development of novel technologies can only be successful if they are transferred and used in the field with a sustainable quality-assured application to allow for the optimal detection and effective control of diseases. The aim of these new tools is to detect the presence of a pathogen agent before the onset of disease. This manuscript focuses mainly on the experiences of two World Organisation for Animal Health collaborating centers in context to molecular diagnosis and molecular epidemiology of transboundary and endemic animal diseases of viral origin, food safety and zoonoses (Expert Review of Molecular Diagnostics (2009), 9: 367-381).

#### Prevalence of *Brucella* abortus antibodies in serum of Holstein cattle in Cameroon.

P.H. Bayemi, E.C. Webb, M.V. Nsongka, H.Unger and H.Njakoi

Holstein cattle of a small scale dairy production system were screened for Brucella abortus antibodies in 21 villages in Cameroon by ELISA. Results show a general seroprevalence of 8.4% in Holstein cattle. Of the 192 cows tested, 14 were infected giving a within-sex seroprevalence of 7.3% while 6/74 bulls were infected with a seroprevalence of 8%. There was no evidence (P=0.11) of differences in seroprevalence between age groups although animals above one year and below three years accounted for nearly half of the infected animals. 64% of infected animals were found in three locations (P=0.015): Kutaba (32%), Bamendankwe (16%) and Finge (16%). A specific control programme should be organized at these locations. Measures should be taken to ensure the eradication of the disease within the population and sound control measures adopted to avoid a further spread of the disease to larger cattle populations. Infected animals should be slaughtered systematically. All farmers should be advised to boil milk before consumption. Vaccination against Brucella abortus should be instituted and use of artificial insemination propagated. In order to ensure a productive and healthy population of Holstein cows within the dairy production scheme, regular Brucella testing should be instituted (Tropical Animal Health and Production (2009) 41:141-144).

# **Recently Published**



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

The aim of this manual is to provide a comprehensive guide to the methods involved in collecting, preparing and screening plants for bioactive properties for manipulating key ruminal fermentation pathways and against gastrointestinal pathogens. The manual will better equip the reader with methodological approaches to initiate screening programmes to test for bioactivity in native plants and find 'natural' alternatives to chemicals for manipulating ruminal fermentation and gut health. The manual provides isotopic and non-isotopic techniques to efficiently screen plants or plant parts for a range of potential bioactives for livestock production. Each chapter has been contributed by experts in the field and methods have been presented in a format that is easily reproducible in the laboratory. It is hoped that this manual will be of great value to students, researchers and those involved in developing efficient and environmentally friendly livestock production systems. The book will be released in early 2010.

# Managing Prenatal Development to Enhance Livestock Productivity

Prenatal life is the period of maximal development in animals, and it is well recognised that factors that alter development can have profound effects on the embryonic, foetal and postnatal animal. Scientists involved in research on livestock productivity have for decades studied postnatal consequences of foetal development on productivity. Recently, however, there has been a surge in interest in how to manage prenatal development to enhance livestock health and productivity. This has occurred largely due to the studies that show human health in later life can be influenced by events during prenatal life, and establishment of the Foetal Origins and the Thrifty Phenotype Hypotheses. This book, Managing the Prenatal Environment to Enhance Livestock Productivity reviews phenotypic consequences of prenatal development, and provides details of mechanisms that underpin these effects in ruminants, pigs and poultry. The chapters have been divided into three parts:

- Quantification of prenatal effects on postnatal productivity;
- Mechanistic bases of postnatal consequences of prenatal development; and
- Regulators of foetal and neonatal nutrient supply.

Managing the Prenatal Environment to Enhance Livestock Productivity is a reference from which future research to improve the level of understanding and capacity to enhance productivity, health and efficiency of livestock in developing and developed countries will evolve. It is particularly timely given the development of molecular technologies that are providing new insight into regulation and consequences of growth and development of the embryo, foetus and neonate.

The book has been released in November 2009.

## Veterinary Diagnostic Real-time PCR Handbook

This book gives a comprehensive account of the practical aspects of real time PCR and its application to veterinary diagnostic laboratories. The optimisation of assays to help diagnose livestock diseases is stressed and exemplified through assembling standard operating procedures from many laboratory sources. Theoretical aspects of PCR are dealt with as well as quality control features necessary to maintain an assured testing system. The book will be helpful to all scientists involved in diagnostic applications of molecular techniques, but is designed primarily to offer developing country scientists a collection of working methods in a single source. The book is complimentary to the Molecular Diagnostic PCR Handbook published in 2005. The book will be released in early 2010. 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

#### **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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January 20F€

The APH Newsletter is prepared twice per year by the Animal Production and Health Section, Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture and FAO/IAEA Agriculture and Biotechnology Laboratory, Seibersdorf.

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