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Scientific Forum "Food for the Future. Meeting the challenge with nuclear applications". 18-19 September 2012, Vienna, Austria.

To Our Readers

Dear Colleagues,

It is with gratitude that I thank you all for your cooperation, loyalty and support to the Animal Production and Health Subprogramme of the Joint FAO/IAEA Division during 2012. This was indeed a momentous year, with food insecurity, food safety issues, production failures and price increases threatening the stability and sustainability of food and agriculture in many countries and regions. I hope that our interactions will continue to grow as each year passes-by and with each newsletter. I especially want to express my appreciation to all who have provided feedback on our Coordinated Research Projects (CRP), our Technical Cooperation Projects (TCP) and the numerous other activities highlighted in past newsletters and on our web page. We hope that this will continue.

As 2012 draws to an end, I want particularly to highlight the IAEA Scientific Forum on "Food for the Future: Meeting the Challenges with Nuclear Applications", held during the IAEA General Conference (18–19 September 2012). The Forum focused on the multitude of challenges faced by farmers in many of our Member States due to fragile food and agriculture environments. In the following, I want to share with you some of the highlights and concerns from the Scientific Forum. Having focused on water and water resources in 2011, the Director General of the IAEA decided to give priority in 2012 to another major global challenge, namely that of global food insecurity.

IAEA International Atomic Energy Agency

Thus, the 2012 Scientific Forum examined the challenges related to the improvement of food production, food protection and food safety through the use of nuclear applications.

In the first session on "Increasing Food Production" it was noted that the world will need to produce 70% more food between now and 2050 to satisfy the demand of a population in excess of 9 billion people. In this regard, the intensification and diversification of more and higher quality food in a climate-smart and sustainable manner whilst protecting the environment is therefore critical to smallholder farmers and is the key to poverty reduction and increased food security. The Forum noted that the increasing global population faces the challenge of substantially increasing food production under conditions of severe land degradation that has led to a significant reduction in the productive capacity of agricultural lands. Sustainable soil management, plant mutation breeding and animal nutrition and production are therefore critical to the improvement of agricultural productivity.

Group of panelists answering questions from the audience during the Scientific Forum "Food for the Future", held in Vienna, Austria in September 2012.

The second session on "Ensuring Food Protection" noted that global food insecurity is inherently linked to pests and diseases that harm or kill livestock and crops, as well as people working in rural agricultural areas. The losses caused by diseases and pests at both the pre- and post-harvest levels average 30–40% of agricultural outputs, making returns on agricultural investments in land, seeds, water, fertilizer, animal feed, labour and other inputs correspondingly inefficient. In addition, the world is currently facing an unprecedented increase of invasive animal and plant diseases and pests that threaten food security by causing serious losses in production and by necessitating costly control measures, including the escalating use of increasingly expensive pesticides. Outbreaks of secondary pests, the development of resistance of pests to pesticides and the increasing threat of zoonotic diseases to public health cause serious barriers to national and international trade and major losses in export revenues. Nuclear techniques, developed and transferred by the IAEA, provide effective, target-specific and environment-friendly animal and plant pest and disease control methods, thus contributing to food security by reducing production losses, production costs and the need for agrochemicals, thereby overcoming sanitary and phytosanitary barriers to international trade in agricultural products. It was further noted that the laboratories of the Joint FAO/IAEA Division have an important role in the development and dissemination of nuclear methodologies that efficiently manage or defeat crop diseases and pests, and that the development of early and rapid, conventional and advanced diagnostic technologies to Member States should be further expanded

In the third session, on "Enhancing Food Safety", it was noted that the IAEA plays a key role in the development of systems for the control of chemical contaminants in food, in the application of traceability systems to identify and manage emerging food safety problems and trends, and in the provision of information on food origin and authenticity that can help ensure food safety throughout the entire food production chain. It was also noted that food irradiation, strongly supported by the IAEA, is a proven and effective post-harvest treatment to improve food safety and maintain quality through the reduction of bacterial contamination and for the control of insect pests in agricultural commodities, without the need for chemicals or additives. The panelists further noted that food irradiation is one of the few technologies to address both food quality and safety, and that applications of food irradiation for sanitary (human health) and phytosanitary (plant health) purposes helps ensure food safety and quality and facilitate international trade, while at the same time generating significant foreign exchange through the export of food produce.

The Scientific Forum highlighted the substantial capabilities in nuclear sciences and technologies that have been established in numerous Member States. However, more still needs to be done to optimize these in the endeavours to further improve global food security. I will keep you updated on progress from our side as we address these challenges moving forward.

On the staff front, the Subprogramme welcomed the following staff members.

Mohammed Shamsuddin as our "Livestock Reproductionist and Breeder". Mohammed is from Bangladesh and obtained his degree in veterinary medicine from the Bangladesh Agricultural University and his PhD from the Swedish University of Agricultural Sciences in Uppsala. His knowledge and experience in theriogenology will certainly move our reproduction and breeding activities forward.

Bharani Settypalli as a consultant in charge of developing technologies for multiple animal disease diagnosis. Bharani, is from India and obtained his PHD in bio-

technology from the University of Guru Jhambeshwar, India.

Abel Wade as a consultant to work on the molecular epidemiology of ASFV strains circulating in Central Africa. Abel is from the Laboratoire National Vétérinaire (LANAVET) in Garoua, Cameroon and obtained his veterinary medicine degree in Maiduguri (Nigeria).

William Dundon as a consultant to work on peste des petits ruminants (PPR). William is from Ireland and holds a PhD in microbiology from Trinity College in Dublin.

Richard Kangethe as a consultant for the development of irradiated vaccine for the control of trypanosomosis. Richard, a Kenyan national, has recently obtained his PhD in biochemistry at the University of Kwazulu Natal.

Wu Xu is from the Animal Science College, Fujian Agriculture and Forestry University, China, and joined our laboratory as a cost free expert for a period of six months. He is working on animal genetics, especially on the radiation hybrid panel mapping of the goat genome and the genetic characterization of indigenous animal breeds using molecular markers.

Both past, present and future activities are described in further detail on our website and I strongly encourage you all to visit it and to let us know your ideas, comments, concerns or questions. We thank all those who have responded to our request to update the details of their contact and mailing addresses, and urge others to do so by informing Roswitha at R.Reiter@iaea.org.

We have to say farewell to Eszter Fesus who joined us as project assistant in July 2011. Her support in organizing our international meetings is highly appreciated and we will miss her.

Finally, I wish you and your families all the best in the year ahead.

Gerrit Viljoen,
Head, Animal Production and Health Section

Staff

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

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– 2012 Highlights –

The Animal Production and Health Subprogramme focuses and prioritizes its research works on technology development and transfer by applying nuclear and related techniques in assisting Member States to improve livestock production and health. This is achieved through the improvement of animal management practices, reproduction and breeding, disease prevention and control measures, and efficient utilization of locally available feed resources. The Subprogramme divides its activities in three major pillars:

- Optimal use of locally available feed resources.
- Reducing risk from transboundary animal diseases (TADs) and those of zoonotic importance.
- Molecular technologies for improving productivity in smallholder livestock systems.

Project team members in a field-day with Maasai farmers in Arusha, Tanzania.

One of the major highlights in 2012 was the Subprogramme participation in the IAEA Scientific Forum on "Food for the Future: Meeting the Challenges with Nuclear Applications", held at the IAEA in September. The forum was organized by the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture and focused on challenges related to food production, food protection, and food safety through the use of nuclear applications. More than 800 delegates participated over the 2-day event.

Under the regular FAO/IAEA Programme, six Coordinated Research Projects (CRP) are being implemented. Four CRPs are in animal health, which are (1) Early and sensitive diagnosis and control of PPR, (2) Use of irradiated vaccines, (3) Stable isotopes to trace bird migrations in relation to the highly pathogenic avian influenza and (4) Molecular and nuclear technologies for the control of foot and mouth disease. The two CRPs in animal production are (1) Gene-based technologies in livestock breeding and (2) Use of enzymes and nuclear technologies to improve the utilization of fibrous feeds and reduce greenhouse gas emissions from livestock. In total, 76 research contracts holders in 38 developing countries and 21 agreement holders in 15 countries are working in their respective networks to develop improved tools, adapt modern technologies and find out solutions to improve livestock productivity and income of rural farmers and peasants. This R&D network is a valuable chain to keep track of animal production and health activities.

The Subprogramme is also running various projects funded by external donors for research work at IAEA Seibersdorf's laboratories, for strengthening disease diagnostic laboratories in Africa and for implementing training courses in various countries. As part of these activities, APH staff members have delivered expert services in several African countries for coordination of project activities and development of laboratories. The Subprogramme has been working hand in hand with FAO and OIE headquarters in Rome and Paris, respectively.

Eight regional training courses were conducted this year with the participation of 26 internationally recruited lecturers and 168 trainees. The training courses were:

- Regional (AFRA) Training Course on Molecular Diagnostic for Transboundary Animal Diseases held in Uganda in April, 2012.
- Regional Training Course on Early and Rapid Nuclear and Nuclear-Related Diagnostic and Tracing Technologies for African and Classic Swine Fever held at IAEA Seibersdorf's Laboratories, Austria in May 2012.
- Regional Training Course on Molecular Diagnostics for Transboundary Animal Diseases held in China in July 2012.
- Regional Training Course on Molecular and Serological Methods Applied to Diagnosis and Surveillance of Transboundary Diseases" held in Garoua, Cameroon in July 2012.
- Regional Training Course on Early and Rapid Nuclear and Nuclear-Related Diagnostic and Tracing Technologies for West Nile Fever, Hepatitis E and Equine Infectious Anaemia held in Turkey in October 2012.
- Regional Training Course on Artificial Insemination in Small Ruminants held in Tunisia in October 2012.
- Regional (AFRA) Training Course on Molecular Epidemiology and Bioinformatics in TADs Surveillance held in Kenya in November, 2012.
- Regional Training Course on Molecular Methods Applied to Diagnosis, Epidemiology and Surveillance of Transboundary Animal Diseases" held at IAEA Seibersdorf's Laboratories, Austria, December 2012.

The Subprogramme provided technical support to 46 national Technical Cooperation (TC) projects in 2012. Among them, 10 TC projects were implemented in Asia,

one in Europe, five in Latin America and 31 TC projects in Africa. In addition, there are five ongoing regional TC projects with the participation of 75 countries. One regional TC in Africa deals with the diagnosis and control of transboundary animal diseases, one in the European-Asian region on the control of transboundary animal diseases with socioeconomic impact and that affect human health, one in Asia on early warning, response and control of transboundary animal diseases, one in the Arab-Asian region on improving the reproductive and productive performance of local small ruminants by implementing reliable artificial insemination programmes, and one in Latin America on integrated control of fascioliasis in support of national programmes that is being completed.

Efficient feeding coupled with artificial insemination increases milk yields and conception rates.

Capacity building is an important component of TC projects and Subprogramme activities. Regional and national training courses play an important role for the transfer of knowledge and on the improvement of skills of project team members. The individual or group training provided through fellowships and expert missions are of high value in the accomplishment of specific tasks such as setting up laboratory techniques, devising quality control programmes, understanding the use of complex equipment, developing research protocols, implementing field activities, etc. For this purpose, 27 fellowship training and 4 scientific visits were completed and another 60 are expected to be completed by the end of the 2012–2013 TC cycle. It is worth mentioning that 42% of the trainees were female scientists.

International experts and IAEA technical officers visited 23 project sites on 30 travel missions. Individual missions usually last for five working days. On a few occasions, local training courses are implemented to take advantage of the visit of the experts. Travel reports prepared by the experts are valuable documents because they describe facilities available at host institutions, progress made, needs for equipment and training, activities to be implemented within a given timeline by project teams and highlights major constraints that may jeopardize success.

Trainees at IAEA Seibersdorf's laboratory learning immunological and molecular techniques for disease diagnosis.

Three Consultants Meetings (CM) were organized. The first one was on Applying good laboratory practices in molecular testing of multiple diseases in veterinary laboratories, the second one on How to detect and quantify inefficient use of nutrients in livestock production systems: the role of nuclear and isotopic techniques and the third one on Early and rapid veterinary diagnostic tools. All the meetings were held at the IAEA in Vienna, Austria. Invited scientists of high international reputation in their respective fields of expertise participated in these meetings and presented the state-of-the-art methodologies and techniques that can be used, validated, tested, or adapted in developing countries for the improvement of livestock productivity and prevention and control of animal diseases. Generally, some outputs of these meetings are directly applied in TC projects but most commonly they are the foundations for future CRPs or contribute to reinforce ongoing CRP activities.

The Subprogamme will continue its efforts in the development and transfer of nuclear and related technologies to IAEA Member States towards achieving global food security by improving livestock productivity and livelihoods of smallholder farmers and peasants in the context of changing climate.

Forthcoming Events

2nd RCM of the CRP entitled Genetic Variation on the Control of Resistance to Infectious Diseases in Small Ruminants for Improving Animal Productivity (D31026)

Technical Officers: Mohammed Shamsuddin and Kathiravan Periasamy

The IAEA Coordinated Research Project (CRP) entitled Genetic Variation on the Control of Resistance to Infectious Diseases in Small Ruminants for Improving Animal Productivity has been running since 2010. The overall objective of this CRP is to improve productivity in smallholder livestock production systems by using gene based and related technologies.

The second RCM is scheduled to take place from 11 to 15 February 2013 in Bogor, Indonesia. The purpose of the meeting is to review progress achieved in sample and data collection for the phenotypic evaluation of local goat and sheep breeds in relation to genetic resistance to internal parasitism, to evaluate preliminary data on genotyping of DNA samples and to devise a detailed work plan for individual research contracts for the final phase of the programme.

2nd RCM of the CRP entitled The Use of Enzymes and Nuclear Technologies to Improve the Utilization of Fibrous Feeds and Reduce Greenhouse Gas Emission from Livestock (D31027)

Scientific Officer: Nicholas Odongo

The CRP entitled The Use of Enzymes and Nuclear Technologies to Improve the Utilization of Fibrous Feeds and Reduce Greenhouse Gas Emission from Livestock has been running since 2010. The overall objective of this CRP is to coordinate research designed to improve the retention of fiber and the efficiency of milk production in livestock in developing countries, where the available feed resource base is characterized by scarce and fluctuating quantity and quality.

The second RCM is scheduled to take place from 13 to 16 May 2013 at the IAEA in Vienna, Austria to review the results from the several studies conducted during the first 18 months of the CRP. Work plans and activities planned for the remaining 236 months of the CRP will be revised and updated. This will include in vivo evaluation of best-bet candidate fibrolytic enzymes on ruminal fermentation, microbial protein synthesis, microbial diversity and populations, methane production, nutrient intake, diet digestibility, and milk production and composition.

2nd RCM of the CRP entitled Development of Molecular and Nuclear Technologies for the Control of Foot and Mouth Disease (FMD) (D32028)

Scientific Officer: Gerrit Viljoen

The CRP entitled Development of Molecular and Nuclear Technologies for the Control of Foot and Mouth Disease (FMD) has been running since 2010. The overall objective of this CRP is to develop guidelines and protocols which support more effective quality control of FMD vaccines and their application in FMD endemic countries as part of the FAO program for the global progressive control and eventual eradication of FMD in domesticated animal reservoirs will be developed.

The second RCM is scheduled to take place from 8 to 12 April 2013 at FAO Headquarters in Rome, Italy.

Regional (ARASIA) training course on Advanced Molecular Genetic Tools for Characterization and Improvement of Indigenous Small Ruminants (RAS/5/063)

Technical Officer: Kathiravan Periasamy

The training course is part of the activities of TC Project RAS/5/063 Improving the Reproductive and Productive Performance of Local Small Ruminants by Implementing Reliable Artificial Insemination Programmes. The course is addressed to scientists from ARASIA Member States and has the aim to enhance knowledge and capacity building of participants on practical applications of advanced molecular genetic tools for evaluation, characterization, and genetic improvement of indigenous small ruminants.

Participants will be trained in using different molecular genetic platforms that upon implementation will result in improved efficiency in applying advanced tools for genetic characterization of livestock, improved capacity to analyze and manage large scale genotypic data, improved ability to use genome sequence information for the identification of DNA markers associated with economic traits, and better ability for conservation decision making on animal genetic resources (AnGR). The training course will be held at FAO/IAEA Agriculture and Biotechnology Laboratory, in Seibersdorf, Austria from 17 to 21 June 2013.

Past Events

Regional (ARASIA) training course on Artificial Insemination in Small Ruminants (RAS5063)

Technical Officer: Mario Garcia

The training course was held at the École nationale de médecine vétérinaire (ENMV), Sidi Thabet, Tunisia from 1 to 5 October 2012, as part of the TC ARASIA RAS/5/063 activities. The aim of the training course was to provide knowledge and know-how on sheep and goat reproductive physiology, reproductive management and selective breeding strategies. Lectures were conducted at ENMV and laboratory sessions, semen collection and processing, and artificial insemination (AI) procedures were done at the National AI Centre, which was close to the host institute. The Course Director was Dr Naceur Slimane.

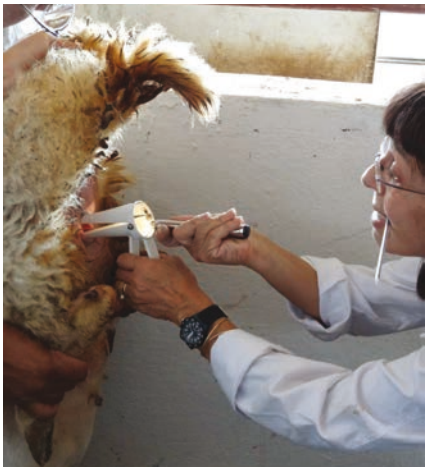
Training course participants.

The course was attended by 31 participants from Algeria, Cameroon, Chad, Egypt, Iraq, Jordan, Kuwait, Lebanon, Oman, Sudan, Tanzania, Tunisia and Yemen. Sixteen participants were financially supported by the Arab Atomic Energy Agency – AAEA. Dr Maria Dattena (Italy), three ENMV staff members and the Technical Officer participated as lecturers and implemented the practical work in the semen laboratory and in the farm.

Lectures and practical work included:

- Reproductive physiology including oestrous cycle and seasonality in goats and sheep.
- Assessing reproductive status by ultrasonography and endoscopy, overview of radioimmunoassay (RIA) and enzimeimmunoassay (EIA) techniques for measuring hormones.
- Manipulation of the oestrous cycle: heat detection and heat synchronization.
- Management and training of semen donors; semen collection, evaluation and processing; semen storage and distribution.
- Artificial insemination techniques for goats and sheep: vaginal, cervical, trans-cervical and laparoscopic; equipment needed.
- AI system: sire selection, animal identification and recording performance data, basic training for AI technicians.

Practical session on laparoscopic artificial insemination in small ruminants.



Examination of the cervix prior to artificial insemination using fresh diluted semen in a ewe.

Lecturers presented up to date information related to AI in small ruminants, including selection and rearing of potential semen donors, semen collection and processing, semen evaluation, transport of semen to the field, training of AI technicians, and equipment needed in the laboratory and in the field to deliver an effective AI service. All participants had the opportunity to practice on evaluation semen quality, semen processing and preparation of semen straws for AI. Also, all participants did clinical and gynaecological examinations of goats and ewes and

were able to practice insemination by the vaginal and cervical methods.

Consultants meeting on Applying Good Laboratory Practices in Molecular Testing of Multiple Diseases in Veterinary Laboratories

Technical Officer: Adama Diallo

This meeting was held from 16 to 18 October 2012. The meeting discussed the applications of appropriate molecular diagnostic technologies and platforms suitable for veterinary testing laboratories with minimum resources, recommendations on practical implementation of GLP conditions, the multidisciplinary approach, core facility and sustainability in veterinary diagnostic and testing laboratories of Member States. Experts from Member State laboratories discussed the current status of molecular tools being implemented and the further measures to be taken in the successful approach for better disease management and experts from The National Veterinary Institute & SLU (Uppsala, Sweden), CIRAD-INRA (France), Institute of Tropical Medicine Antwerp (Antwerpen), AHVLA (New Haw Surrey, UK), Laboratoire de Virologie, ISRA/LNERV (Senegal), Instituto Zooprofilattico Sperimentale delle Venezie (Italy), CSIRO Livestock Industries (Australia), National Centre for Veterinary Diagnostics (Vietnam), National Institute of Animal Health(Thailand), FAO (Rome, Italy) discussed the guidelines needed to be implemented.

3rd RCM of the CRP entitled The Early and Sensitive Diagnosis and Control of Peste des Petits Ruminants (PPR) (D32026)

Scientific Officers: Adama Diallo and Hermann Unger

The CRP on "Early and Sensitive Diagnosis and Control of Peste des Petits Ruminants", launched in 2007, was run from 2008 to 2012. The final RCM took place from 19 to 22 November 2012 at the IAEA in Vienna and IAEA's Nuclear Sciences and Applications Laboratories in Seibersdorf. The overall objective of this CRP was to develop, validate and transfer to Member States sensitive, specific and rapid tests for the diagnosis of Peste des petits ruminants (PPR). The purpose of that final meeting was to assess the work done during the past 5 years in the frame of this project. It was attended by all but one contract and agreement holders.

PPR is a viral disease of small ruminants and is widely spread in Africa, the Middle East and Asia. An example is the recent PPR outbreak in the Democratic Republic of Congo where more than 20 000 goats died from the disease in a few months.

Group picture of RCM participants.

The presentations and discussions during this 4-day meeting can be summarized into three topics: diagnostic tests, epidemiology, and vaccine.

Regarding the diagnostic tests, the quantitative RT-PCR assay for the detection of PPR virus (PPRV) developed at CIRAD was evaluated by different contract holders. The assay was proved to be very sensitive. A similar test was developed at the Animal Production and Health Laboratory (APHL) of the IAEA. This assay is considered as an improvement of the former one since it includes an internal control. Another assay developed at the IAEA for the detection of PPRV is the one based on the loop mediated isothermal amplification, LAMP. It is highly sensitive. A demonstration of the test with a prototype kit was made at the RCM. It was already field tested in Cameroon. It proved to be a technology which can bring a dramatic improvement in the control of PPR by allowing an early detection of the disease on spot. Two commercial kits are currently available for the serological diagnosis of PPR. Both of them have a cross-reaction with rinderpest antibodies. Although rinderpest has been eradicated worldwide, it is still important to make tests available that are strictly specific to PPR. In that direction, a potential breakthrough was reported at this RCM: indeed, a new test is developed based on the use of a PPR recombinant antigen that is not recognized by rinderpest antibodies. This test, which is being developed at the African Union/PANVAC laboratory in Ethiopia, is still in the stage of the proof of concept. Another major advance made during this project was the development of a recombinant tissue culture cell line expressing the goat SLAM protein that facilitates the isolation of PPRV from pathological samples. This new cell line is highly efficient for isolating wild type PPRV from pathological specimens. It is being successfully used by some of the project partners.

The epidemiology of PPR was further advanced through serosurveillance in the respective countries on different animal species. The results for sheep and goats were similar to those already reported in the literature: 20 to 45% samples tested positive for PPR antibodies. One of

the most important results of these studies is the increase of PPR seroprevalence in cattle: from 10 to 20%, even 30% in a country. It looks like the eradication of rinderpest has left "the place" for PPR infection in cattle. Even though cattle are considered dead end hosts for PPRV, the continued infection of cattle may constitute a risk for this species. In line with this fear, participants at the meeting recalled the report on a case of PPR in domestic buffaloes in India in 1997 and a successful reproduction of rinderpest-like disease in calves inoculated by PPR-infected materials, an experiment carried out in Senegal in 1956. However there is no indication yet that cattle can play a role in the epidemiology of PPR. But camel, another domestic animal species in which PPR antibodies were found in some countries, may be involved in the epidemiology of PPR. Indeed, during a study carried out in the Sudan, PPRV was recovered from infected camels. The virus was also successfully transmitted to small ruminants that were placed in contact with experimentally infected camels. Based on this report, it was highly recommended to continue the study of PPR infection in camel. Another subject was the rapid expansion of the disease in Africa, in particular in the southwards direction. Sequence data obtained from 2008 to 2012 show the presence of PPRV lineage IV in Africa with its expansion southwards, while lineage II is expanding westwards, replacing lineage I in Côte d'Ivoire, Burkina Faso and Senegal.

In the field of vaccines the successful development of a PPRV infectious clone from a cDNA representing the full genome of the virus was announced. This indicates that an important step has been made towards the development of a PPRV vaccine that would enable the differentiation between infected and vaccinated animals, the so-called DIVA vaccine. A brief report was made also on the use of short interference RNA (siRNA) for the inhibition of PPRV replication in infected cells and the potential use of this technology as an antiviral treatment.

In conclusion, all participants lauded this PPR CRP as a very successful project with interesting and important achievements. However there is a clear need for further studies to validate the new serological ELISA tests and its application for camels and the application of gene sequencing technology, for the rapid identification of new PPRV strains for the understanding of the current change in the geographical distribution of PPRV lineages.

Considering the importance of the results achieved so far and the will of the international communities to launch programmes for the control or even eradication of PPR, the participants recommended that the IAEA either extend the current project or initiate a new one on PPR.

The participants (contract and agreement holders) were: Mr Chowdhury Emdadul Haque (Bangladesh), Mr Sidibe Mamadou (Burkina Faso), Mr Ngangnou André (Cameroon), Mr Li Gang (China), Mr Bodjo Sanne Charles (Africa Union, Ethiopia), Mme Libeau Geneviève (France), Mr Otsyina Hope Richard (Ghana), Mr Shamaki David (Nigeria), Mr Couacy Emmanuel (Côte d'Ivoire), Khan Qaiser Mahmood (Pakistan), Mr Yahia Hassan Ali (Sudan), Mr Merza Malik (Sweden), Unsal Baca Aysel (Turkey).

Consultants meeting on How to Detect and Quantify Inefficient Use of Nutrients in Livestock Production Systems: the Role of Nuclear and Isotopic Techniques

Technical Officer: Nicholas Odongo

This meeting was held from 19 to 21 November 2012 at the IAEA, Vienna, Austria. The purpose of the meeting was to brainstorm on nutrient use efficiency in livestock production systems and come up with conclusions and recommendations on (i) how to detect and quantify inefficient use of nutrients in different livestock production systems, (ii) how to close this efficiency gap to maximize resource use, and (iii) the role for nuclear and isotopic techniques. Three participants from France and from FAO attended this meeting.

The meeting was attended by Dr Paulo Cortes Salgado from CIRAD - La Reunion Island, Dr. Eliel González García from INRA - Montpellier, Mr. H. Makkar from FAO, HQ, Rome, and Mr. M. Shamsuddin and Mr. N. Odongo from the Joint FAO/IAEA Division.

Group picture of meeting participants.

Although the experts recognized and highlighted the importance of using a holistic approach to enhancing nutrient use efficiency encompassing improving reproductive efficiency, animal health, animal welfare and overall management of resource use, these were considered outside the scope of the meeting. Some of the key conclusions and recommendations of the meeting were the following:

- Better feed inventory at the farm, regional and national levels (what, when and how much is available including the seasonality of available) feed resources. This information is important for sound planning of the livestock sector and for developing supplementation and conservation strategies.
- "If you don't measure it, you can't manage it"..... Peter Drucker. Improving the accuracy of quantifying what and how much (voluntary feed intake) the animals are consuming, and the partitioning of nutrients thereof, is crucial for understanding and managing nutrient use efficiency.
- Better characterization of feed composition and allocation of resources at a farm and regional level so that low quality feed goes to maintenance stock, e.g. dry cows, and higher quality feed goes to higher nutrient requirement physiological states, e.g. young growing animals, lactating and late pregnant animals. This ensures minimal feed processing or treatment and most efficient use of nutrients.
- Need to promote forage production (by using good quality seeds and good agronomic practices) to decrease the cost of feeds and/or reduce the reliance on imported feed ingredients (including total mixed rations) to meet the animal requirements, including the conservation of local biodiversity resources.
- Proper ration balancing will ensure efficient use of nutrients in animal food-chain and will reduce wastage, GHG emissions and nutrient loading into the environment and/or nutrient deficiency in the animal.
- Better understanding of the rumen ecology and functions will contribute to improve rumen efficiency and therefore animal productivity. 454 pyrosequencing and DGGE techniques will enable identification of rumen microbe; understand their diversity and link ecology to rumen function.
- Better understanding and determination of nutrient requirements of local breeds will contribute to increase the efficiency of utilization of available feed resources.
- Better understanding of water use efficiency and characterization of feeding systems at regional and national levels.

Training course on Molecular Methods (PCR and Sequencing) Applied to Diagnosis, Epidemiology and Surveillance of Transboundary Animal Diseases

Technical Officer: Adama Diallo

The training course took place from 10 to 21 December 2012 at the IAEA's Nuclear Sciences and Applications Laboratories in Seibersdorf, and focused on PPR, CBPP, AI, ND, FMD and Capripox. The training was conducted by the International Atomic Energy Agency (IAEA) and the Food and Agriculture Organization (FAO) of the United Nations through the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, the Animal Health Service (FAO-AGAH) and the FAO Emergency Centre for Transboundary Animal Diseases (ECTAD) regional units for Eastern Africa and Western and Central Africa to promote the use of Gene based identification and classification of pathogens by veterinary diagnostic and research laboratories of Africa. The course was attended by 20 scientists from veterinary institutes and diagnostic laboratories of Nigeria, Senegal, Ethiopia, Botswana, DRC, Tanzania, Cameroon, Mali, Kenya and Uganda. Well-recognized experts delivered lectures and demonstrated the principles and practical applications on genetic analysis of animal pathogens. Hands on training with practical laboratory sessions on well established procedures harmonized for gene amplification, sequencing and sequence analysis was provided.

First RCM of the CRP entitled Use of Stable Isotopes to Trace Bird Migrations and Molecular Nuclear Techniques to Investigate the Epidemiology and Ecology of the Highly Pathogenic Avian Influenza (D32030)

Scientific Officer: Ivancho Naletoski

The CRP D32030 was approved in 2012. The CRP team comprises 2 agreement holders (scientific advisors), 3 technical contractors (laboratories performing advanced laboratory analyses) and 7 research contractors (counterparts responsible for field sampling and initial laboratory tests). The overall objective of the CRP is to establish a scientifically justified platform for non-invasive monitoring of wild bird migrations and evaluation of their role in the long range transmission of avian influenza viruses (AIVs).

The idea of the project was initiated because the increased interest of the global scientific community in the use of stable isotopes (SI) as intrinsic markers for determination of wild bird (waterfowl) migrations. The principle of evaluation is based on the difference in the ratios of these SIs at different geographical locations. As their concentration in animal tissues (especially in the metabolically inert tissues, such as feathers) is strongly related to the food and water intake, it is possible to determine the origin of certain birds or flocks by measuring SI ratios in feathers. The ratios of the ^{2}H (deuterium) are generally considered to be sufficient for evaluation of the long-range migrations. However, as different SIs have different metabolic backgrounds and pathways, combined measurement of multiple SIs may increase the resolution of determination. This phenomenon is intended to be used in combination with the detection of AIVs in faecal samples and determination of the bird species using DNA barcoding technique, also in faecal samples. Therefore, this approach, if scientifically justified will enable wide

epidemiological investigations of the role of migratory wild birds in transmission of AIVs, without the need of bird capturing or hunting. Moreover, this approach, with necessary modifications, may be used in the future for evaluation of the role of numerous wild animal species in transmission of other transboundary animal diseases, such as foot and mouth disease, African and classical swine fever, rabies, African horse sickness blue tongue and others.

Group photo of the counterparts participating at the RCM under the CRP D32030.

During the RCM (31 October–2 November 2012), the counterparts of the CRP agreed on using a harmonized approach for sample collection, labeling and initial testing from the countries of the technical contractors (Egypt, Turkey, Bulgaria, Russian Federation, Tajikistan, Nepal, China and the Republic of Korea). Uniquely labeled samples will be tested in the local laboratories by using same diagnostic techniques, as follows: 1) AIV M-gene detection using Real Time RT-PCR, 2) The RNA from the positive samples will be converted to cDNA, 3) The DNA from the positive and a portion of negative samples will be extracted and used for DNA barcoding and 4) The positive and a portion of the negative samples will be shipped to the IAEA laboratory in Seibersdorf, Austria and to the laboratory of the technical contractor for advanced AIV analyses (AHVLA, Weybridge, UK). In order to maximize the inter-laboratory harmonization, a ring trial will be organized for the AIV M-gene detection and the reverse transcription phase (cDNA production) at the counterparts' laboratories.

Feather samples will be directly send to the laboratory of the technical contractor at Environment Canada for determination of the SI ratios.

The obtained results will be stored in an MS Access database located on a "cloud" server with defined access permissions for each counterpart in the CRP. This database will also be used as an information resource for the counterparts, as it is already filled with multiple on-line links providing information on the biology, ecology and the migrations of wild birds. Periodical review of the data in the database is also planned in order to adjust the future activities towards the most balanced sampling schemes (redefinition of target species, bird categories, locations, etc.).

Regional training course on Early and Rapid Nuclear and Nuclear-Related Diagnostic and Tracing Technologies for West Nile Fever, Hepatitis E and Equine Infectious Anaemia (RER/5/016)

Technical Officer: Ivancho Naletoski

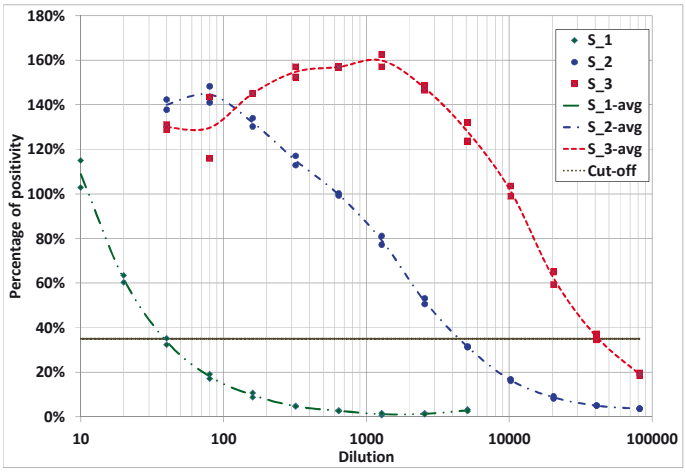
Hands-on exercise in the molecular laboratory.

A training course for early and rapid diagnosis of West Nile Fever (WNF), Equine Infectious Anemia (EIA) and Hepatitis E (HE) was organized at the Bornova Veterinary Control and Research Institute, in Turkey (Host and course director: Dr Fethiye Coven), from 8 to 19 October 2012. The topics of the training course covered application of serological and molecular techniques for diagnosis of the three diseases. A separate component dedicated to the quality control (QC) issues during the performance of the diagnostic tests was provided to the participants.

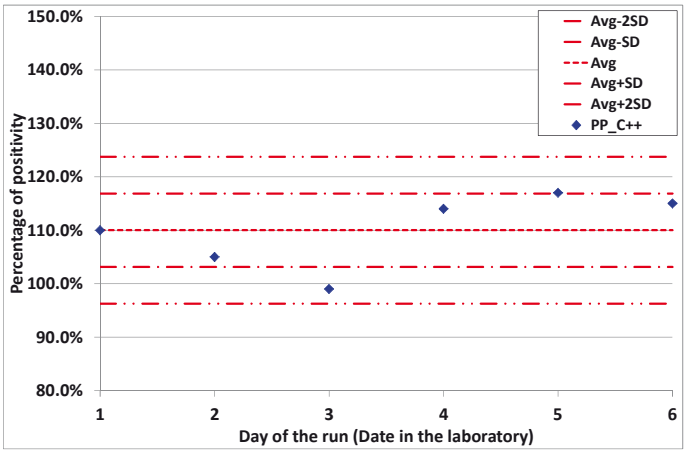
Theoretical lectures on the use of QC sera, held by the IAEA expert.

Four experts participated in the training course as lecturers: Dr Sylvie Lecollinet (WNF) and Dr Aymeric Hans (EIA), both from the EU reference laboratory for equine

diseases, ANSES, France, Dr Frederik Widén (HE) from the National Veterinary Institute in Uppsala, Sweden and an IAEA expert for QC in ELISA techniques, from the IAEA Seibersdorf Laboratories.



Titration of the local standard QC sera (red and green lines) against the referent standard (blue line) in ELISA.



Use of locally produced QC sera for determination of the daily performance (deviation) in ELISA.

Twenty four participants from 13 countries attended the course. The course was organized as hands-on exercises on the use of diagnostic methods to early detect the three diseases. From serological methods, AGID, iELISA and cELISA were demonstrated and from molecular methods, the conventional and the Real Time PCR. The scope of use, the diagnostic performances, as well as the epidemiological importance (interpretation) of each of the diagnostic tests were comprehensively discussed with the experts.

The QC component of the training course covered topics on production of local QC sera for the serological methods (harmonized with appropriate international reference standard), determination of optimal dilutions for the strong and the weak positive controls, as well as the statistical calculations used for the daily monitoring of the test performances. For QC in molecular techniques, calculation and interpretation of the Real Time PCR standard curves, as well as quantification of the DNA amplicons were demonstrated. All the topics of the QC component of the course are of critical importance for implementation and maintenance of the ISO 17025 international standard in the counterpart laboratories, specifically in the area of validation and verification of the diagnostic tests.

Group photo of course participants.

Consultants meeting on Advanced Technologies for Rapid Detection and Characterization of Existing and Emerging Vector-borne Pathogens of Livestock

Scientific Officer: Ivancho Naletoski

The consultant meeting was held at the Headquarters of the Joint FAO/IAEA Division in Vienna, Austria on 11 and 12 December 2012. Six invited speakers from USA (California Animal Health & Food Safety Laboratory System and the Center for Grain and Animal Health Research (CGAHR) of USDA, ARS), Germany (Friedrich-Loeffler-Institut, Island Riems, Germany), France (ANSES Laboratoire de Santé Animale), Spain (Universidad Complutense de Madrid) and the United Kingdom (Animal Health Veterinary Laboratories Agency- Addlestone) gave presentations on the topic of the meeting.

The presentations focused on the use of next generation sequencing, metagenomics in detection of animal pathogens, luminex and DNAChip-based assays, microarrays suitable to detect new and/or related pathogens and other advanced technologies for rapid detection and characterization of existing and emerging vector-borne pathogens of livestock.

A wider audience of internationally recognized reference laboratories were invited to participate at the meeting. Additionally, some of the project counterparts in the projects of the Animal Production and Health Section participated as observers. A more comprehensive meeting report will be published in the next issue of this Newsletter.

First coordination meeting of the Regional project Supporting Early Warning, Response and Control of Transboundary Animal Diseases (RAS/5/060)

Technical Officer: Hermann Unger

The aim of this meeting was to initiate this regional project and to gather information from the participating Member States on TAD's affecting their countries, the problems faced with diagnosis and intervention and to express their view of how to address these in the national and regional context.

The meeting started on July 2 in Lanzhou, China with introductory remarks by Prof. Hong Ying, meeting organizer. The participants from Bangladesh, China, Indonesia, Cambodia, Mongolia, Myanmar, Pakistan, Philippines, Sri Lanka and Thailand presented the specific livestock disease situation and the problems faced with their control or eradication. A summary of diseases affecting the livestock production in the region was drawn up.

Specific presentations on advances in research in China on avian diseases (Avian Influenza, NCD), FMD and PPR were presented by members of the Lanzhou Veterinary Research Institute to give insight into the highly advanced research capacity in the region and to offer perspectives in controlling these diseases. Specific topics resulting from the presentations were addressed, namely problems of information exchange between veterinary services and headquarters, the lack of thermostable vaccines reducing the efforts of blanket vaccination, and rapid disease diagnostics and the need for training in the different areas.

A general work plan for the 4 years duration of the project was drawn up, including the training courses and meetings and individual programmes for country specific issues. Foreseen training courses are on molecular diagnostics with focus on PPR and FMD and will include isothermal techniques; Epidemiology and risk analysis with focus on emerging diseases; Molecular epidemiology for monitoring disease spread; Vaccine technologies; selection criteria/test and production tools. The individual countries expressed their special interests to cover disease of local importance like Hantan virus. The establishment of ring trials for proficiency testing was initiated and should start with FMD diagnosis.

Regional (AFRA) training course on Molecular Epidemiology and Bioinformatics in TADs Surveillance (RAF/5/057)

Technical Officer: Hermann Unger

The training course was held at the Trypanosomiasis Centre at Kari, Nairobi, Kenya from 16 to 20 April 2012. Twenty participants form 15 AFRA countries participated and were instructed by the 2 lecturers from South Africa.

The course started with a review on molecular biology, bioinformatics and functional genomics by Dr Paul Mireji. A general introduction to molecular epidemiology and online bioinformatics tools and free software was given by Dr Markotter. During the second day of the course, important principles in molecular epidemiology, basic principles of PCR and DNA sequencing were defined, and discussions on phylogenetics were introduced.

Practical activities included the use of BioEdit software and its applications, training on sequence analysis using GenBank and BLAST and multiple alignments using Clustal X/ BioEdit by Dr Coertse. Participants were fit to design their own molecular trees and learned how to build a tree from unknown sequence using MEGA 5.

For conventional and real-time Taqman PCR new tools for primer and probe design for PPR diagnosis were performed.

This training will allow the participants and their respective laboratories to improve their molecular diagnostic capacity including extended trouble shouting. At the same time it will be of help for the region as the technologies taught will allow the spatial description of disease occurrence by the analysis of genetic changes. This allows the mapping of disease 'migration' and is important to design appropriate counter measures like quarantine or immunisation exercises.

Regional training course on Molecular Diagnostics for Transboundary Animal Diseases (RAS/5/060)

Technical Officer: Hermann Unger

The IAEA training course for molecular diagnostics took place in Langzhou, China from 15 to 19 October 2012 with 17 participants from 9 Asian countries. The general aim of this course was to improve knowledge about important "cornerstones" of molecular techniques, such as the laboratory structure and the necessary equipment, basic and advanced methods and assay design. The schedule comprised 10 lectures (The Molecular Lab / Protocol to SOP / DNA and RNA / Bioinformatics / PCR

/ Gel Electrophoresis / Quantitative PCR / Detection Formats / Primer Design / LAMP) as well as practical sessions covering PCR, gel electrophoresis, qPCR and LAMP.

Group photo of training course participants.

The introduction and training on LAMP was a determined key point, since this easy and fast approach is expected to potentially replace the standard technology PCR for diagnostic purposes.

An additional aim was to generate a fully interactive working environment. To do so, the laboratory was asked to provide WIFI and all participants were instructed to bring and use laptops. This allowed the participants to simultaneously work with the same online tools, such as netprimer, BLAST and primer-blast, in parallel with the lecturer. The bioinformatics included basic Excel programming leading to the development of a master mix matrix and primer design using the freely accessible tool PerlPrimer. The use of the PCR instrument software allowed shared analysis of the qPCR results, which allowed discussing important settings in greater details.
There were intense discussions for a better understanding of the different topics. The best example was the group work on the qPCR protocol highlighting the need to question provided protocols.

All practical exercises gave the expected results in high quality. It was possible to define standard curves with both one and two step qPCR protocols. The LAMP approach yielded positive results for crude and purified control samples of different pathogens (PPR, NDV, H5N1). The highlight of the amplification approaches admittedly was the use of the outer primers of the PPR LAMP design for all methods.

This training of trainers will help to first of all extend the human capacity base in molecular diagnostics in Member States to establish these technologies as standard diagnostic tools. Specifically the introduction of the isothermal diagnostic techniques into the national veterinary laboratories will allow establishing molecular diagnostics much more rapid in peripheral laboratories and even in the field.

Regional training course on Molecular and Serological Methods Applied to Diagnosis and Surveillance of Transboundary Diseases and also Research Methodology

Technical Officer: Charles Lamien

The Regional Training Course was held at the Laboratoire National Vétérinaire (LANAVET), Garoua, Cameroon, from 23 July to 03 August 2012. The course was jointly funded by the USAID funded "Identify" project and the South Africa-funded "African renaissance fund (ARF)" project. Twenty-one participants from 14 African countries attended the course. Participants were trained on several conventional and real time PCR methods developed at the Animal Production and Health Laboratory of the Joint FAO/IAEA Division for capripoxvirus detection and differentiation.

Participants of the training course at LANAVET.

On the Occasion of the 50th Anniversary of NA Laboratories at Seibersdorf

In 2012 the IAEA's Nuclear Sciences and Applications Laboratories in Seibersdorf commemorated 50 years of dedicated support to Member States in their efforts to address developmental needs through the peaceful uses of nuclear science and technology. These laboratories (8 in total) are a unique feature in the United Nations system, and are responsible for supporting and implementing programmatic activities that respond to the development needs of 158 Member States in food and agriculture, human health, environmental monitoring and assessment, and the use of nuclear analytical instruments. To do so, the laboratories carry out three essential types of demand-driven activities: applied research and development, training and capacity building, and technical and analytical services.

The Animal Production and Health Laboratory (APHL) uses radioisotopes and related technologies to improve reproductive efficiency, map economically important genes to improve animal productivity and genetically characterize indigenous livestock breeds. It is known globally for its role in developing and popularizing molecular and immunoassay methods for diagnosis, control and eradication of many transboundary animal diseases.

IAEA Director General Mr Yukiya Amano and some of the APH staff at the photo exhibition.

The celebration took place on November 2012 in the IAEA Board room. The opening speech was given by the IAEA Director General Mr Yukiya Amano and further remarks were given by HE Ambassador and Chairman of the IAEA Board of Governors Mr John Barrett, HE Ambassador of South Africa Mr Xolisa Mabhongo and the Host Austria, Dr Wolfgang Thill. Mr Iain Darby from Nuclear Instrumentation delivered his speech on behalf of laboratory staff. The video 50 Years of IAEA Labs at Seibersdorf was shown and this can be watched in the IAEA website using the link http://www.iaea.org/newscenter/news/2012/seiblab.html (Serving Humanity, 28 November 2012)

The ceremony included a photo exhibition together with live displays by the NA Seibersdorf Laboratories, where APHL booth presented some of the past and current laboratory activities and the various mechanisms of support to CRPs and TC projects.

View of the Animal Production and Health booth during the photo exhibition during the 50th Anniversary Celebration.

2nd RCM of the CRP entitled The Use of Irradiated Vaccines in the Control of Infectious Transboundary Diseases of Livestock (D32029)

Technical Officers: Adama Diallo and Herman Unger

The second RCM took place from 25 to 29 June 2012 in Nairobi, Kenya. The purpose of this RCM was to assess the results that have been obtained so far and the difficulties that were met in order to develop new work programmes with advice from the agreement holders. This meeting was attended by all Research Contract holders (9) and Agreement holders (3).

The CRP includes studies on *Trypanosoma evansi, Theileria annulata, Ichthyophthirius multifiliis, Brucella abortus, B. melitensis, Fasciola hepatica, F. gigantica and Haemonchus contortus.*
Nearly all Research Contract holders reported results on the determination of the efficient irradiation dose to obtain a non-replicative but metabolic active vaccine:

- For *Fasciola hepatica* the dose of irradiation seems to be low (50 Gy to obtain an attenuation of the metacercariae).
- With *Ichthyophthirius multifiliis*, the pathogen responsible of the White Spot Disease (WSD) of Rainbow trout, the optimum dose range of irradiation for the inactivation of the pathogen was 150–170 Gray.
- For *Theileria annulata*, cell-culture schizonts were used as starting material for the irradiation. Preliminary results showed a dose between 100 and 150 Gy.
- For *Brucella melitensis*, 300 Gy are needed to efficiently inactivate the bacteria.

Preliminary results have shown interesting results in the identification of effective dose for the attenuation of pathogens were reported. In general, irradiation doses are much lower than doses that were used in the past for the pathogen inactivation.

Stories

Somebody is reading our newsletter – Library of Botswana National Veterinary Laboratory.

Establishment of a new tool, Loop Mediated Isothermal Amplification (LAMP), into a useful diagnostic test for Leptospirosis in Sri Lanka (IAEA TC SRL 5/042)

Background

In keeping with the need to expand and improve allied health care services in the country, the Molecular Medicine Unit (MMU) was established by the Faculty of Medicine of the University of Kelaniya, Sri Lanka in March 2002. The MMU provides molecular diagnostic services for infectious/genetic diseases to the general public, promotes research in molecular biological aspects of infectious diseases/genetic diseases and to carryout undergraduate and postgraduate teaching and training activities. Molecular diagnostic assays for number of infectious diseases such as Chikungunya, Dengue, Filarisis, Hepatitis, Leptospirosis, Malaria and Tuberculosis were developed with the help of the IAEA. Most of laboratory equipment and training for the MMU were received by the IAEA. Laboratory is operating fully with Standard Operational Procedures (SOPs) for all selected diseases and radio safety and Quality Assurance and Quality Control (QA/QC) manuals. Significant funding from international and governmental organizations was obtained to continue and develop the project, most of which was after the upgrading of laboratories and training provided by the Agency. Numerous national and international collaborative links were established as well as to veterinary institutions.

Under the current IAEA TC SRL 5/042 project titled "Molecular diagnosis of zoonotic infections in Sri Lanka", a new tool, Loop Mediated Isothermal Amplification (LAMP) was established as a useful diagnostic test for Leptospirosis under the supervision of Dr. H. Unger of the IAEA/FAO.

Introduction

Leptospirosis is one of the most important zoonotic diseases in the world, caused by pathogenic bacteria belonging to the genus Leptospira which infect man and animals like cattle, rodents or dogs. It is an emerging and reemerging disease in Sri Lanka. Diagnosis of leptospirosis mainly depends on clinical symptoms and signs in Sri Lanka. Clinical diagnosis may result in over or under estimation of the disease prevalence as these are not specific enough. Therefore, laboratory confirmation of leptospirosis suspected patients is important to measure the real incidence of the disease and proper patient management.

The objective of this study was to establish a simple molecular diagnostic assay which could be used even at a rural hospital with minimum facilities and in veterinary laboratories.

Methodology

The new isothermal molecular tool, Loop Mediated Amplification (LAMP) using five oligonucleiotide primers based on a Leptospira specific gene sequence had been previously designed (Lin et al., 2009) and was modified and established in the laboratory to determine its fitness for purpose and the inherent analytical sensitivity and specificity. A panel of blood samples together with clinical information was collected from 150 leptospirosis suspected patients after obtaining written consent. Blood samples from acute cases were collected during 1–5 days of fever and convalescent blood samples were collected after 7–14 days of the collection of acute blood sample. Hundred and six patients having both acute and convalescent samples were included in Group 1. Forty four patients having only acute samples were included in Group 2. Acute serum samples in both Groups 1 and 2 were tested by Polymerase Chain Reaction (PCR) (Gravekamp et al., 1993) and LAMP assay. Convalescent samples in Group 1 were tested by Leptocheck IgM immunochromatography and MAT assays. Chi-squre test or Fisher's exact test were used for comparison of data.

The LAMP assay was as well applied for detection of pathogenic Leptospira in cattle and rats. Collection of urine samples from cattle and whole blood samples from rats from an outbreak area are ongoing. Testing

animal samples by LAMP, real time PCR and/or MAT is also on going. So far 46 urine samples from cattle and 40 blood samples from rats were collected.

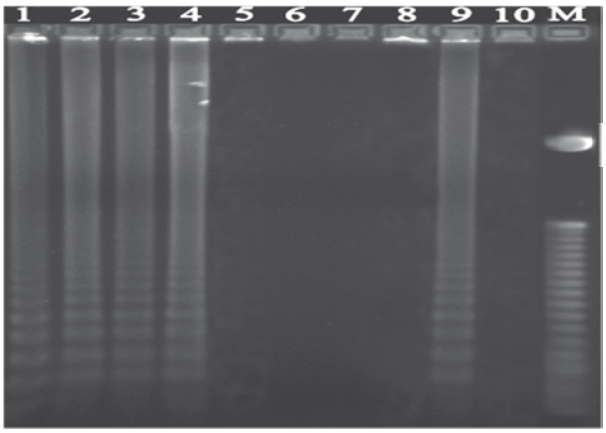
Analysis of field samples at the laboratory established with the help of the IAEA.

Results

The LAMP assay showed a high diagnostic sensitivity of 49 fg/ml. The figure shows typical banding pattern in gel electrophoresis of the LAMP amplicons for clinical samples infected with pathogenic Leptospira collected from the field. In our setting the LAMP assay showed 87% (131/150) positivity while PCR assay gave 19% (29/150) positivity on the same acute clinical samples. This is a significant statistical difference between the LAMP assay and PCR-AGE assay ($\chi2$=136, p=0.000) for diagnosis of leptospirosis patients. There was no significant statistical difference between results of patients in Group 1 confirmed for LAMP assay (on acute serum samples) and Leptocheck IgM immunochromatography (on convalescent samples) ($\chi2$=0.88, p=0.34) and MAT (on convalescent samples) ($\chi2$= 1.89, p=0.15) (Table 1).

In this study, all 150 patients presented with clinical features of leptospirosis but the proportion of laboratory diagnosed leptospirosis patients among the suspected patients were 97% (146/150). On comparison of presence of clinical features used for diagnosis of leptospirosis according to World Health Organization (WHO) criteria, only meningeal irritation showed significant association with leptospirosis infection (1/146 vs 1/4, $\chi2$=3.98, p= 0.048). This indicates importance of laboratory confirmation of leptospirosis in suspected patients.

In application of the Leptospira LAMP assay for urine samples of cattle showed 17% (8/46) were positive for pathogenic Leptospira. Further samples are currently under investigation, as this test was only established later on for urine samples.



Agarose gel picture (1.5%) showing results of LAMP assay for detection of Leptospira in PCR confirmed clinical samples. Lanes 1-4 = DNA from clinical samples positive by PCR, Lanes 5-8 = DNA from clinical samples negative by PCR, Lane 9 = DNA from L. interrogans (Positive control), Lane 10 = Negative/reagent control (DW), Lane M = 50 bp DNA molecular weight marke.r

Agarose gel picture (1.5%) showing results of LAMP assay for detection of Leptospira in PCR confirmed clinical samples. Lanes 1–4 = DNA from clinical samples positive by PCR, Lanes 5-8 = DNA from clinical samples negative by PCR, Lane 9 = DNA from L. interrogans (Positive control), Lane 10 = Negative/reagent control (DW), Lane M = 50 bp DNA molecular weight marker

Table 1. Results of laboratory diagnostic assays for leptospirosis

		No. of positives		
Type of sample	Type of assay	Group 1 (n=106)	Group 2 (n=44)	Total
Acute (n=150)	LAMP (+)	87% (92/106)	89% (39/44)	87% (131/150)
	PCR-AGE (+)	21% (22/106)	16% (7/44)	19% (29/150)
Convalescent (n=106)	Leptocheck IgM kit (+)	49% (52/106)		
	MAT (+)	34% (36/106)		

Conclusion

The project has made an excellent progress in meeting the objectives of establishing and assessing the new tool at the MMU. This LAMP assay which shows high analytical sensitivity and specificity can be used for early, definitive and rapid diagnosis of human leptospirosis using a single blood sample even in a remote hospital with minimum facilities. This type of study on development of a rapid diagnostic assay have a significant effect for rapid confirmation of outbreaks and limit spread of such outbreaks from one geographical area to another. Further, rapid confirmation of leptospirosis

outbreaks will reduce the socio-economic impact on individuals and government.

The successful engagement of the government, disease control program managers and clinicians from the outset of the project has laid the foundations for sustainability and for moving the technology into the clinical setting. Provision of molecular diagnostic services for leptospirosis to the general public is another outstanding goal achieved under the project. The project has also generated an expertise which can be expanded to include this technology for other high burden diseases.

These stories as well as other articles are also available under 'Highlights' on our Homepage
http://www-naweb.iaea.org/nafa/aph/index.html

Coordinated Research Projects

Project Number	Ongoing CRPs	Scientific Secretary
D3.10.26	Genetic variation on the control of resistance to infectious diseases in small ruminants for improving animal productivity	Mohammed Shamsud-din
D3.10.27	The use of enzymes and nuclear technologies to improve the utilization of fibrous feeds and reduce greenhouse gas emissions from livestock	Nicholas Odongo
D3.20.26	The early and sensitive diagnosis and control of peste des petits ruminants (PPR)	Adama Diallo
D3.20.28	The control of foot and mouth disease (FMD)	Gerrit Viljoen
D3.20.29	The use of irradiated vaccines in the control of infectious transboundary diseases of livestock	Adama Diallo
D3.20.30	Use of stable isotopes to trace bird migrations and molecular nuclear techniques to investigate the epidemiology and ecology of the highly pathogenic avian influenza	Ivancho Naletoski

The control of foot and mouth disease (FMD)

Technical Officer: Gerrit Viljoen

The FMD CRP investigates vaccine matching procedures, vaccine potency testing methods and guidelines, and procedures by which an FMD vaccine's ability to induce production of protective antibodies in cattle without the need for animal challenge experiments can be evaluated.

The first Research Coordination Meeting (RCM) of the Coordinated Research Project (CRP) on The Control of Foot and Mouth Disease, FAO, Rome, Italy, from 10 to14 January 2011, was held in collaboration with FAO and EU-FMD. It was attended by all, but one, research contract holders and agreement holders as well as several observers from EU-FMD and FAO and Foot and Mouth (FMD) vaccine and diagnostic manufacturers and producers. Discussions were focused on (1) the status of FMD in the participating counterpart's respective countries (e.g. FMD free *vs.* FMD free zone with or without vaccination *vs.* FMD endemic) with respect to the risks and threats; (2) what are currently being done in terms of vaccine matching; (3) what criteria are being used to choose FMD vaccines and how they are being applied; (4) how are vaccine potency being determined and utilized; (5) how are post-vaccination monitoring and surveillance being performed; (6) the status of counterpart's vaccine laboratory quality assurance and FMD laboratory analysis and diagnoses (i.e. their analysis and/or diagnostic laboratory proficiencies and capacities both for routine testing and research, laboratory infrastructure and procedures). The work plans of all the research contract holders (RCH) and the agreement holders (AH) were developed and discussed and all the agreement holders will supervise (based on their respective expertise) identified aspects of the work plans.

Foot and mouth disease 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 they 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 1980s, but these are not standardized for international use. Many countries now

produce FMD vaccines but often without proper consideration of their effectiveness.

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).

All the counterparts developed their work plans such that, individually and or collectively, they work towards generating solutions set by the objectives of the FMD CRP.
It is important to:

- Establish methods and develop internationally agreed protocols for measuring the potency of FMD vaccines using *in vitro* methods;
- 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;
- 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;
- Provide further global coordination of current research into FMD vaccines for use in endemic settings and to cooperate with other FMD institutions such as EU-FMD and PANAFTOSA;
- To evaluate and standardize:
    - Virus neutralization (VN) tests,
    - Early and rapid lateral flow and dip-site technologies and their application and use,
    - Antigenic cartography (at IAH and OVI) in relation to virus neutralization tests (VN).

The second RCM will take place from 8 to 12 April 2013 at FAO Headquarters in Rome, Italy.

The use of enzymes and nuclear technologies to improve the utilization of fibrous feeds and reduce greenhouse gas emissions from livestock

Technical Officer: Nicholas Odongo

Implemented under the IAEA project 2.1.2.1 entitled Integrated Management of Animal Nutrition, Reproduction and Health, this CRP has the overall objective to improve the efficiency of using locally available fibrous feed resources to improve livestock productivity while protecting the environment. The CRP was initiated in September 2010 with the award of eleven Research Contracts, three Research Agreements and one Technical Contract. The First RCM of the CRP was held 7-11 February 2011 in Lethbridge, Alberta, Canada and it was attended by all 15 Contract and Research Agreement Holders and one observer.

The first activity of the CRP was for all research contract holders to conduct baseline surveys to characterize the various fibrous feed resources available in the project area, e.g. rice and wheat straw, maize stover, tropical grass, bagasse and sugarcane tops etc. and to establish how much of these were available and how they are currently being used. During the survey, samples of the available feed resources were collected for chemical compositional analysis in the laboratory and sub-samples stored for later in vitro and in sacco evaluations. Most contract holder have completed the compositional analysis and are currently evaluating enzymic activities of candidate enzymes. All research contracts were renewed for the 2012/2013 fiscal year.

In the current phase, contract holders will be (i) finalizing the chemical compositional analysis of various fibrous feed resources collected during the survey, (ii) determining the major enzymic activities of candidate exogenous fibrolytic products (xylanase PLUS and cellulase PLUS), (iii) evaluating the two enzymes (xylanase PLUS and cellulase PLUS) in vitro and in situ using locally available substrates at none, low, medium, high dose rates and (iv) using batch culture incubations for in vitro screening of candidate exogenous fibrolytic products in buffered rumen fluid to determine their effects on 24 and 48 h NDF degradation.

The second RCM is scheduled to take place from 13 to 16 May 2013 at the IAEA in Vienna, Austria.

Genetic variation on the control or resistance to infectious diseases in small ruminants for improving animal productivity

Technical Officer: Mohammed Shamsuddin

The objective of the CRP is to improve productivity in smallholder livestock production systems by using gene based and related technologies. The specific objectives are:

- To develop capacity in developing countries in the use of molecular and related technologies and create opportunities for international research collaboration;
- 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;
- To collect phenotypic data and DNA samples from goat and sheep breeds or populations within breeds with a history of infectious disease resistance;
- To develop expertise on the use and development of bioinformatic tools for the analysis of large datasets of genomic data related to parasite resistance in various breeds;
- To provide valid data for the identification of genetic markers associated with infectious disease resistance and to initiate the development of tools for molecular diagnostics and assisted breeding.

Twelve research contract holders from Argentina, Bangladesh, Brazil, Burkina Faso, China, Ethiopia, Indonesia, the Islamic Republic of Iran, Nigeria, Pakistan, Saudi Arabia and Sri Lanka and four agreement holders from Brazil, Italy, Kenya and the United States of America are currently participating in the CRP.

The first part of the experimental work, the artificial challenge with infective L3 *Haemonchus contortus* larvae to animals representing resistant and susceptible breeds to quantify the relative resistance to gastrointestinal parasites has been completed in most of the participating countries, DNA has been extracted and some RC holders have sent subsets of DNA to the Animal Production and Health Laboratory in Seibersdorf, Vienna. Most contract holders are currently working on a large field trial using at least 500 animals of a single breed collecting information related to body weight, FEC, PCV, and FAMACHA scores. Blood samples for DNA analysis are also being collected. Both the experimental challenge and the field trial were completed by the end of 2012.

The first research coordination meeting was held in Vienna, Austria, from 21 to 25 February 2011, and the second RCM will be hosted by the Bogor Agricultural University from 11 to 15 February 2013 in Indonesia.

The early and sensitive diagnosis and control of peste des petits ruminants (PPR)

Technical Officer: Adama Diallo and Herman Unger

Peste des petits ruminants (PPR) is a highly contagious transboundary animal disease of wild and domestic small ruminants caused by a morbili virus similar to Rinderpest virus and is on the list of economically important animal diseases to be reported to the OIE. PPR spread in endemic regions through nomadic herdsmen and livestock trade. High morbidity and mortality rates up to 90% in affected herds make PPR a killer disease for small ruminant populations. This not only affects rural economies severely but also reduces the genetic resources and endangers breeding policies. Clinically, PPR is characterised by high fever, depression and anorexia followed by ocular and nasal discharge, pneumonia and severe diarrhoea. These symptoms can easily be confounded for pasteurellosis or rinderpest and diagnostic tests for RP were giving positive results due to the cross reactions.

The disease is endemic in parts of Africa, the Near and Middle East and South Asia and the incidence is gradually expanding and the Animal Production and Health Subprogramme receives regular requests from Member States for support. In Africa, PPR was limited to countries north the equator but since 2007 Gabon, Democratic Republic of Congo, Kenya, Angola, and Uganda amongst others reported outbreaks. The situation is similar in former Soviet Union countries of Asia. In addition, PPR is one of the targets of the United Nation Food and Agriculture Organisation's (FAO) Emergency Preventive System (EMPRES) programme.

It is not easy to isolate PPRV in cell culture and might need up to 2 to 4 weeks for a positive result to be confirmed. In the late 1980's specific reagents (monoclonal antibodies) and nucleic acid techniques (DNA probe hybridization and polymerase chain reaction, PCR) became available and allowed more precise diagnosis. Today different ELISA's and PCR procedures are in use. However, these techniques are evolving quickly and need constant adaptation. An APH organized consultancy meeting in 2007 on the Early and rapid diagnosis of emerging and re-emerging transboundary animal diseases concluded that "amplification systems, in the form of real-time PCR (rt-PCR) as well as isothermal amplification (IA) approaches have moved from research environments to routine diagnostic applications". The APH Subprogramme was encouraged to foster the transfer of these new technologies to IAEA and FAO Member States. Their application of early and sensitive PPR diagnostic tools, in combination of protective and DIVA (differentiation between infected and vaccinated) vaccines to PPR would improve our management and control of the disease.

The overall objective of the CRP is to develop, validate and transfer to Member States sensitive, specific and rapid tests for the diagnosis of Peste des petits ruminants (PPR) to help them better manage and control this TAD.

The specific research objectives are:
(a) Evaluate and validate current Reverse Transcriptase-PCR (RT-PCR) methods in use for the diagnosis of PPR;
(b) Evaluate and validate real-time PCR;
(c) Design and evaluation of the loop-mediated isothermal amplification (LAMP) assay;
(d) Evaluate and validate a penside test currently under development for rapid and cheap identification of PPR virus in the field;
(e) Evaluate and validate the use of ELISA in epidemiological studies of disease prevalence and protection due to vaccination;
(f) Contribute to build up PPRV gene sequence data bank for molecular epidemiology analysis.

Eleven Research Contract holders from Bangladesh, Burkina Faso, Cameroon, China, Ghana, Cote d'Ivoire, Mali, Nigeria, Pakistan Sudan and Turkey, and four Agreement holders from Australia, Ethiopia, France and Sweden participated in the CRP.

The final RCM was held 19 to 22 November 2012 at the IAEA in Vienna and IAEA's Nuclear Sciences and Applications Laboratories in Seibersdorf.

The use of irradiated vaccines in the control of infectious transboundary diseases of livestock

Technical Officer: Adama Diallo

Many of the vaccines used today rely on technologies developed over 100 years ago involving some form of attenuation, i.e. the use of an alternative or mutant strain of a pathogenic organism that has reduced virulence whilst maintaining immunogenicity, or inactivation, where chemical or physical methods are used to kill virulent pathogenic strains. Amongst the success stories where control has been achieved can be included smallpox and Rinderpest, two diseases that had a global impact, but have now been eradicated.

For some viral and bacterial diseases there are good vaccines, but for many parasitic and helminth diseases there are limited control measures. Parasitic infections, including tick-borne diseases, animal trypanosomoses, and helminthoses also have a significant impact on productivity causing poor growth, low calving and reduced milk yield. Historically, chemotherapeutic drugs have been the mainstay of treatment and control of diseases caused by animal parasites. They have been viewed as cheap, safe and effective, although of course they are required to be constantly administered to ensure animals will thrive. Their greatest drawback has been in the emergence of drug resistance that reduces their efficacy, or prevents their use; in contrast, there is no evidence that similar genetic adaptation to vaccine-induced immunity ever occurs.

With protozoal infections, a few vaccines have been produced based mainly on live parasites that result in a low level infection that stimulates a protective immune response similar to that produced by the natural infection. These methods include vaccination with low doses of infective organisms (*Eimeria*), infections controlled by chemotherapy (*Theileria parva*), attenuated vaccines (*Babesia, Theileria annulata*) or truncated life cycles (*Toxoplasma*). Multicellular helminth parasites such as trematodes (e.g. *Fasciola, Schistosoma*), nematodes (e.g. *Haemonchus*) and cestodes (e.g. *Taenia*) present an even greater challenge to the development of suitable vaccines since they are large, complex multicellular organisms that, unlike smaller organisms, cannot be internalised by the hosts' phagocytic immune mechanisms. Not surprisingly, few vaccines have been developed to protect against infections with helminth parasites. There is however one commercially available vaccine, against the lungworm *Dictyocaulus viviparus,* consisting of radiation-attenuated, infective L3 larvae. Nevertheless, research to develop other irradiated vaccines was not seriously pursued for the past twenty years.

There is now good reason to reevaluate the use of irradiation attenuation for vaccine production. The recent successful development of an irradiated vaccine for human malaria has demonstrated anew the feasibility and practicalities of this technique and indicated that technical problems can be overcome using existing knowledge without recourse to sophisticated technology.

The general objectives of the CRP are:
(a) To develop protocols for the attenuation of animal pathogens and to define parameters for their use as vaccines against the causative agents of transboundary parasitic and other infectious diseases;
(b) To evaluate the effect of radiation on the potency of the irradiated products;
(c) To examine the potential of irradiation to inactivate viruses as an alternative to chemical treatment.

Eleven Research Contract holders from Argentina, China, Ethiopia, Georgia, India, Islamic Republic of Iran, Kenya, Sri Lanka, Sudan, Thailand and Turkey, and four Agreement holders from Belgium, China, UK and USA are currently participating in the CRP.

The first RCM was held from 11 to 15 October 2010 in the Vienna International Centre, Vienna, Austria, and the second RCM took place from 25 to 29 June 2012 in Nairobi, Kenya.

Use of stable isotopes to trace bird migrations and molecular nuclear techniques to investigate the epidemiology and ecology of the highly pathogenic avian influenza

Technical Officer: Ivancho Naletoski

Among several important issues in the epidemiology of the Highly Pathogenic Avian Influenza (HPAI) that need attention is the role Wild Water Fowl (WWF) populations might play in the dissemination of infection. Tracing the movements of WWF in relation to where they originated as well as their stopover points during their migration between breeding and non-breeding grounds is a particularly challenging task.

It is necessary to utilize methods that can be used on a larger scale and not biased to initial capture location if we are to fully comprehend the role of migratory birds in the spread of avian influenza. A suitable technique that has already been used to trace migrants is based on the stable isotope (SI) signatures of tissues of birds, especially those in feathers. Of most interest are deuterium (δD) ratios in tissues that reflect those in surface (lakes, rivers, oceans) and in ground waters. Since hydrogen isotope composition of environmental water varies spatially across the globe in a predictable manner and its presence relayed to feathers, δD analyses of feathers provides a way of linking SI data on water isoscapes with those in the feathers.

Faecal samples will be used for the detection of AI viruses eventually present in the faeces and extraction and analysis of somatic DNA to detect the bird species. These two techniques will be used to link the AI carrier status and the carrier species, without even capturing the birds, and may thus be used as a non-invasive platform to generate important epidemiological information on migration pathways (obtained by SIA) and the transmission of the virus to certain geographical area. Fecal samples should be collected randomly at the same sites where feathers are collected. Samples will undergo two test procedures:

(a) DNA barcoding (species identification), adapted at the Avian Disease Laboratory; College of Veterinary Medicine; Konkuk University, South Korea. The technique is based on detection of a short gene sequence from a standardized region of the genome as a diagnostic "biomarker" for species. The target sequence has been the 648-bp region of the mitochondrial gene, cytochrome C oxidase I (COI), already optimized as a DNA barcode for the identification of bird species. The optimization of a DNA barcoding technique for faecal samples has been performed by comparing DNA from the faecal samples with the DNA from tissue samples (muscle, feather, and blood) from already known bird species (domestic poultry and WWF), collected from live-bird markets, Conservation Genome Resource Bank for Korean Wildlife, and from the Seoul Grand Park Zoo. The results of bird species identification, using COI gene sequences from tissues matched the faecal samples of the same individuals.

(b) Detection of the AIV in the faecal samples using optimized protocol in five phases: i) detection of M gene to detect the presence of influenza A viruses using PCR technique (Positive samples should be inoculated in SPF eggs for virus isolation), ii) Positive samples should be tested using H5 or H7 protocol on PCR, iii) H5 and H7 positive samples should undergo molecular pathotyping (cleavage site sequencing), iv) M gene positive and H5 and H7 negative, should be further typed in order to differentiate the subtype using conventional (HI-test) and/or molecular methods, v) Positive samples and a portion of negatives will be tested using Loop Mediated Isothermal Amplification (LAMP) protocol.

The main pathway of AIV transmission is faecal contamination. Natural water reservoirs are the media where WWF faeces is excreted in the water and contaminating it randomly. However, the survival of the AIV in the natural water reservoirs depends on numerous environmental, physical and chemical influences, as well as on the period between excretion by an infected and infection of a healthy WWF. Testing of natural water reservoirs will generate information on the level of (eventual) contamination and the risk of AIV transmission via these media at different geographical and environmental conditions. Water samples should be collected from different points of each selected area, in an amount of approximately 500 ml per sample. Each sample should be tested for the presence of AIV, using PCR with previous concentration of the virus. Using a standardized protocol, it is possible to quantitatively evaluate the level of contamination, based on comparison with known titrated virus isolate.
Of great epidemiological interest would be potential application of the same technology to trace short-range migrations in wildlife carriers, in order to determine their role in transmission of animal and/or human pathogens.

Seven Research Contract holders from Bulgaria, China, Egypt, Nepal, Russian Federation, Tajikistan and Turkey, two Agreement holders from Germany, and 3 technical Contract holders from Canada, Republic of Korea and UK are currently participating in the CRP.

The first RCM was held at the IAEA from 31 October to 2 November 2012.

General information applicable to all Coordinated Research Projects

Submission of Proposals

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

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

Complementary FAO/IAEA Support

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

For further information please contact: Svetlana Piedra-Cordero (s.piedra-cordero@iaea.org)

Activities of the Animal Production and Health Laboratory

ANIMAL GENETICS

Genetic variation on the control or resistance to infectious diseases in small ruminants for improving animal productivity

Gastro-intestinal (GI) parasitic infestations incur huge economic loss to poor and marginal farmers rearing sheep and goat across the world. Breeding programmes with the goal of enhancing host resistance to parasites will help to alleviate this problem in the long term. The CRP D3.10.26 'Genetic variation on the control of resistance to infectious diseases in small ruminants for improving productivity' was initiated in 2010 with the objectives of (i) quantifying the level of genetic resistance of indigenous sheep/goat breeds against GI nematodes through artificial challenge and field trials (ii) exploring genetic variation of candidate genes involved in immune pathways among indigenous sheep/goat breeds and (iii) studying the association between genetic variants and level of genetic resistance against GI nematodes. Most of the twelve countries participating in the project have completed the artificial challenge trial and generated phenotypic data on levels of immune response in their native breeds. Animal Production and Health Laboratory at Seibersdorf has been involved in detection of novel single nucleotide polymorphic (SNP) markers, development of competitive allele specific PCR based SNP assays and genotyping of samples received from challenge experiments conducted in member states.

(i) Genetic polymorphisms of candidate genes involved in immune pathways

Initially, a total of 60 SNPs were identified from 49 candidate genes involved in various immune pathways, of which 58 SNPs qualified for the development of genotyping assays. All the genotyping assays were subjected to in-house validation for their suitability to genetic diversity and association studies. Among these, ten SNPs were found to be monomorphic in a panel of 32 unrelated animals during the initial validation process. In order to delineate the underlying genetic diversity of different indigenous sheep breeds with respect to these candidate genes, 370 animals belonging to 14 sheep breeds (Bergschaf, Karakachanska, Krainer-Steinschaf, Shumenska,Texel, Wild Mouflon, Hamdani, Shal, Bangladeshi, Indonesian Fat tailed sheep, Indonesian thin tailed sheep, Junin, Corriedale and Pampinta) were

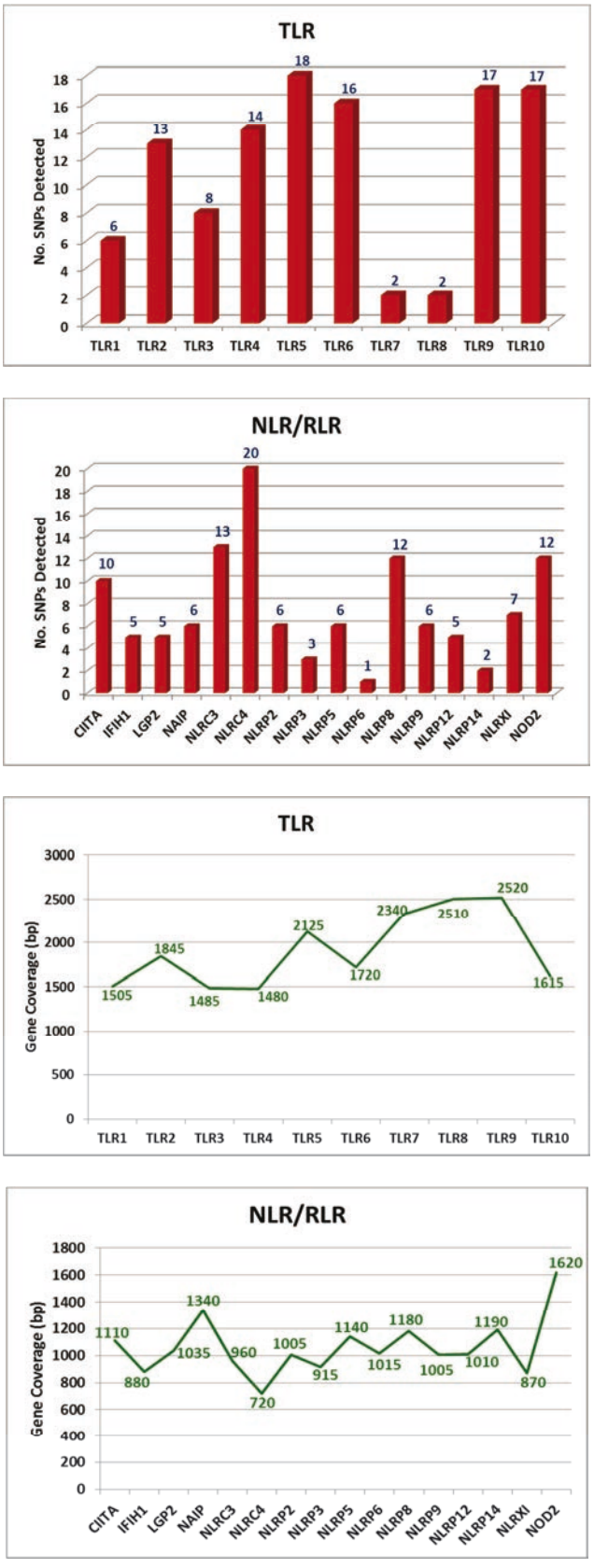


Figure 1. Number of SNPs detected and gene coverage for screening in each of TLR (above) and NLR/RLR (below) genes.

genotyped at all the 58 SNP loci. Genotyping of 347 more animals belonging to 9 indigenous sheep breeds (Madras Red, Mecheri, Pattanam, Nellore, Corriedale, Thalli, Kachi, Karakul and Kajli) are in progress. Meta-analysis of genotypic data from all the 22 indigenous sheep breeds will help to establish the immune gene diversity based genetic relationship of sheep across Asia, Europe and Latin America. Further, genotyping of DNA samples from experimentally challenged animals from four sheep breeds have been completed and six other breeds are in progress, the results of which will be utilized for genetic association studies.

(ii) Genetic variation of pattern recognition receptor genes involved in innate immunity

Pattern recognition receptors (PRR) are surface receptors involved in the host innate immune system and recognize microbe specific molecules called pathogen associated molecular patterns (PAMPs). Some of the PRRs include Toll like receptors (TLRs), NOD Like Receptors (NLRs), RIG-I like Receptors (RLRs), C-type Lectin Receptors (CLRs). Genetic variations within many pattern recognition receptor genes have been found to be significantly associated with genetic disease resistance of various livestock species. However, information on genetic polymorphisms within many of these PRR genes is limited in sheep and goats. Identification of novel SNPs within the candidate genes of different PRRs will be helpful for establishing their underlying genetic variations as well as for genotype-phenotype association studies. At Animal Production and Health Laboratory, 71 sets of primer pairs were designed to screen 10 TLR, 14 NLR, 2 RLR and 15 CLR genes to detect novel SNPs in a panel of eight unrelated sheep. An average of 1.914 kb coding region of each of the ten TLR (TLR1-10) genes was screened and 113 SNPs were detected. In case of NLRs and RLRs, an average of 1.062 kb of each of the 16 genes was screened to detect 119 SNPs (Figure 1). Among the newly detected SNPs, 34 SNPs from TLR genes and 25 SNPs from NLR/RLR genes qualified for assay development. PCR optimization and sequencing for all the 15 CLR genes is under progress.

(iii) Radiation hybrid panel mapping of goat genome

Although goat is an important livestock species, genomic information available on them is relatively poor as compared to other major species such cattle and sheep. For example, the resolution of goat gene map available till date is very low with only few markers being mapped to each of the 30 pairs of chromosomes. The animal production and health laboratory (APHL) in collaboration with other institutions initiated the development of radiation hybrid panels for gene mapping in goats. A 5000 rad goat–hamster whole-genome radiation hybrid (WG-RH) panel was generated and preliminarily characterized. Development of WG-RH panel will provide a framework map for comparative mapping and for assembly of the goat genome sequence. APHL initiated the process of mapping candidate genes related to gastro-intestinal resistance and those involved in various immune pathways including pattern recognition receptor genes on goat genome. Genotyping of RH panel is being atttempted through three different approaches viz., conventional PCR-Agraose gel electrophoresis, competitive allele specific PCR based end-point genotyping and high throughput genotyping using Integrated Circuit Fluidic Dynamic Array System. About 140 gene markers including 92 candidate genes involved in various immune pathways, 10 TLRs, 14 NLRs, 2 RLRs, 15 CLRs and 7 MHC genes will be genotyped. Of these, optimization of genotyping protocols for more than 100 gene markers have been completed. Apart from the candidate gene marker, about 250 microsatellite markers have been identified for mapping the goat RH panels. PCR-Agarose gel electrophoresis and Fluidic Dynamic Array system will be used for genotyping microsatellite markers on goat RH panel.

Genetic characterization of indigenous chicken breeds in search of unique variation in immune related genes

Indigenous chicken populations around the world possess wide genetic diversity and search for beneficial mutations across important immune related genes in them can be helpful for improving bird resistance to diseases. The Myxo virus resistance gene (Mx) is one of the important candidate genes with respect to genetic resistance to Avian Influenza. A total of 610 birds from 18 chicken populations including 13 indigenous breeds (7 from Europe and 6 from Asia) and 5 commercial strains were screened for nucleotide variations at different SNP loci within Mx gene. Apart from the diversity analysis of different chicken breeds with respect to Mx gene variations, the complete Mx gene of ~21 kb size was sequenced in 47 chicks belonging to 13 indigenous breeds across Europe and Asia. PCR amplification and sequencing was done in 16 overlapping fragments to form the contiguous sequence of complete gene. Further analysis of sequence data to assess the difference in chicken breeds from temperate (Europe) and tropical (Asia) locations is under progress.

Global Reference Genetic Repository of livestock breeds for animal genetic research

Global reference genetic repository at Animal Production and Health Laboratory collects preserves and maintains genomic DNA from distinct breeds of various livestock and poultry species including cattle, sheep, goat chicken, alpacas, rabbits, etc. The main objective of establishing genetic repository is to encourage and strengthen the collaborative animal genetic research across different countries. The genetic repository is constantly strengthened by addition of new DNA samples. A total of 275 samples including 245 from 8 breeds of sheep (Mecheri, Madras Red, Pattanam, Nellore, Thalli, Kacchi, Karakul

and Kajli) and 30 samples from Alpacas, rabbits and wild boars were added to the repository during the last six months.

ANIMAL HEALTH

Tools for the rapid detection and differentiation of capripoxviruses

The development of tools for capripoxvirus (CaPV) differentiation is essential to allow the assignment of each field isolate into one of the 3 CaPV groups, sheep poxvirus (SPPV), goat poxvirus (GTPV) and lumpy skin disease virus (LSDV), is necessary because they can cross infect and produce the disease in sheep, goat or cattle. Previous achievements by AHPL are the development of a classical PCR to differentiate SPPV from GTPV/LSDV, two real time PCR methods to differentiate the three CaPVs using fluorescently labeled probes. Current development is to toward real time PCR methods based on high resolution melting and non-labeled probes to produce cost effective methods for easy adoption in veterinary diagnosis and research laboratories of member states.
An assay using saturating dyes and a label-free probe based on Snapback primer technology is currently being finalized for CaPVs differentiation (Figure 1).
The assay was proven to be well suited for genotyping and is very specific to CaPVs. Over 60 isolates from various geographical origins were accurately genotyped and assigned into one of the 3 CaPV groups.

Molecular Epidemiology of Capripoxviruses

The APHL is contributing to the better understanding of CaPV epidemiology by investigating new outbreaks as part of it current research work on capripox or upon request from member states. Recently, on the request of counterparts from Kenya and Ethiopia samples from recent outbreaks in the country were characterized using the capripox virus genotyping tools that were developed at APHL. In addition, two genes were amplified, cloned and sequenced for each isolate. This work has confirmed our first observations of the cross-infection of sheep by GTPV.

Another work undertaken was the molecular survey of various capripox vaccines used in Africa in collaboration with the Pan African Veterinary Vaccine Center. The application of real time PCR and the sequencing of two genes from each of the 11 vaccines that were investigated have shown the predominant use of cattle strain (LSDV) for the immunization of both small ruminants and cattle. Although this confirms the cross protection by CaPVs, further study is needed to understand if this practice should not be reconsidered, especially for some specific capripox which emerge from vaccinated flocks.

E-Learning

The Joint FAO/IAEA division together with the FAO-HQ in Rome and the Swiss Institute of Bioinformatics launched an e-learning module on Phylogenetics of animal pathogens: basic principles and applications.
The vast diversity of the pathogens affecting livestock demands a very specific diagnostic procedure in identification and characterization of each pathogen. In this context, the enormous amount of sequence and genotype data is being generated on animal pathogens, which is further useful in understanding their pathogenicity and molecular epidemiology. The usage of this data in developing efficient molecular diagnostic tools needs basic understanding of the phylogenetic analysis. Phylogenetic classification, construction of trees, interpretation unveils the geographical distribution and migration of pathogens which helps in better management of animal diseases. The e-learning tool was designed with introduction to phylogenetics, tools, building and interpreting trees and finally its application to veterinary diagnostics, with aim of the better management of animal diseases by preparing laboratory technicians, veterinarians and molecular epidemiologists from diagnostic and research laboratories of developing FAO and IAEA member states, to be self-sufficient in the data analysis by interpreting the phylogenetic trees and their relationships. (http://viralzone.expasy.org/e_learning/partners.html)

INTERNS and FELLOWS

- Mr Matthew Clarke, a student at the University of Edinburgh, completed an internship in APHL from 1 June to 31 July, 2012.

- Ms Chinchuluun Boldbaatar from the Institute of Veterinary Medicine, Mongolia was associated with APHL for two months from 3 September 2012 to 31 October 2012 to study advanced molecular diagnostic techniques, and designing and implementing the experiments.

- Ms Kassedo Nina Benedicte Toily from Ministry of Agriculture and Fisheries, Cote d'Ivoire joined the APHL as a fellow on 5 November 2012 for four months.

Technical Cooperation Projects

TC Project	Description	Technical Officer(s)
ALG/5/027	Strengthening Animal Health and Livestock Production to Improve Diagnostic and Reproductive Capacities in Animal Breeding and Support Expertise for the Feasibility Study of a Biosafety Laboratory, Level 3 (BSL3) **Objective:** To contribute to the improvement of animal health and livestock production by using nuclear and nuclear related technologies to strengthen reproductive and diagnostic capacities in animal breeding; to support expertise for the feasibility study of a bios	M. Shamsuddin / I. Naletoski
ANG/5/010	Characterizing Indigenous Animal Breeds for Improving the Genetic Quality of Local Cattle Breeds and Small Ruminants **Objective:** To undertake phenotype and genotype characterization of indigenous animal breeds for improving the genetic quality of local and adapted cattle breeds	M. Shamsuddin
BEN/5/006	Improving Animal Health and Productivity **Objective**: To strengthen, diagnose, and control African swine fever, and increase animal productivity.	H. Unger / A. Diallo
BEN/5/007	Soil, Crop and Livestock Integration for Sustainable Agriculture Development Through the Establishment of a National Laboratory Network **Objective:** An interdisciplinary project has been developed that aims at a sustainable intensification of peri-urban agricultural production through the integration of cropping-livestock systems.	N. Odongo / G. Viljoen
BKF/5/011	Improving the Health and Productivity of Small Ruminants through Efficient Animal Feeding, Identification of Genetic Markers for Breeding Programmes and Better Health and Reproductive Management **Objective:** To improve small ruminants productivity through efficient use of local plant resources in animal feeding and health, identification of genetic markers for using in breeding programmes and better health and reproductive management	M. Shamsuddin
BOH/5/001	Reducing the Incidence of Brucellosis in Animals and Humans by Surveillance and Control **Objective:** To reduce the incidence of brucellosis in animals and humans in Bosnia and Herzegovina	I. Naletoski
BOT/5/005	Improving Diagnosis of Animal Diseases **Objective**: To employ nuclear molecular diagnostic techniques for improved diagnosis of transboundary animal diseases, such as foot and mouth disease, contagious bovine pleuropneumonia, and avian influenza.	G. Viljoen
BUL/5/012	Developing and Validating Molecular Nuclear Technologies for Rapid Diagnostics of Foot and Mouth Disease and Genotyping of Indigenous Cattle Breeds **Objective**: To improve livestock by rapid diagnosis and effective control of foot and mouth disease, and genotyping of indigenous cattle breeds through development and validation of molecular nuclear methodologies.	I. Naletoski / G. Viljoen
BZE/5/004	Strengthening the Veterinary Diagnostic Laboratory with Capacities in Polymerase Chain Reaction Diagnosis (Not funded) **Objective**: To ensure food security through early detection of H5/H7 avian influenza, and other exotic diseases, and to ensure the capacity for quick response to disease outbreaks with epidemiological surveillance.	G. Viljoen

TC Project	Description	Technical Officer(s)
BZE/5/006	Establishing Early and Rapid Diagnosis of Transboundary Animal Diseases to Support Food Security **Objective:** To establish an early and rapid nuclear/nuclear related serological/molecular diagnostic and control capability for transboundary animal diseases:- Building capacity, strengthening of a national diagnosis and surveillance system for transboundary/zoonotic	G. Viljoen
CAF/5/004	Improving Livestock Production Through Disease Control and Artificial Insemination **Objective**: To improve animal production in the Central African Republic through livestock disease control and improved breeding by use of artificial insemination.	I. Naletoski / M. Garcia
CAF/5/005	Enhancing Livestock Productivity through the Improvement of Selection and Use of Artificial Insemination for Increased Meat and Milk Production **Objective:** Improve cattle productivity by implementing a reliable artificial insemination (AI) programme in the country	M. Shamsuddin
CHD/5/004	Improving Cattle Productivity through Genetic Improvement, Including Artificial Insemination, to Contribute to Reducing Poverty and Combating Food Insecurity **Objective:** Improve the productivity of local cattle breeds by means of artificial insemination	M. Shamsuddin
CMR/5/018	Improving Productivity of Indigenous Breeds and Animal Health **Objective:** Improved productivity of indigenous breeds and animal health.	H. Unger
ELS/5/011	Enhancing Livestock Productivity and Decreasing Environmental Pollution through Balanced Feeding and Proper Manure Management **Objective:** Enhance livestock productivity and decrease environment pollution through balanced feeding and proper manure management	N. Odongo
ERI/5/009	Enhancing Small Scale Market Oriented Dairy Production and Safety for Dairy Products through Improved Feeding and Cattle Management, Higher Conception Rates and Lower Calf Mortality **Objective:** To increase dairy production through improved feeding and cattle management and higher conception rate and lower calf mortality, and improve farmers livelihood in Eritrea	M. Shamsuddin / N. Odongo
ETH/5/017	Improving Livestock Productivity through Advances in Animal Health and Production **Objective:** Improvement of livestock productivity through advances in animal health and production	A. Diallo
HON/5/005	Improving the Nutrition and Health Conditions of Livestock in Order to Increase Productivity and Reproductivity (Phase II) **Objective:** To strengthen and improve livestock production in Honduras.	M. Garcia / N. Odongo / G. Viljoen
IVC/5/032	Establishing Epidemiological Surveillance of Peste des Petits Ruminants (PPR) and Studying Its Socio-Economic Impact on Rural Populations by Developing Diagnostic Tools and Providing Economic Data to Veterinary Services **Objective:** To develop diagnostic tools and provide economic data to assist veterinary services in developing a proper strategy to control peste des petits ruminants in Cote d'Ivoire	G. Viljoen / A. Diallo
KAM/5/002	Using Nuclear and Molecular Techniques to Improve Animal Productivity and Control Transboundary Animal Diseases **Objective:** To improve livestock productivity for food security by integrated management of animal nutrition, reproduction and health which includes: early pregnancy diagnosis for better reproductive management, metabolic profiles in livestock for assessing nutrition	G. Viljoen / M. Garcia

TC Project	Description	Technical Officer(s)
KEN/5/027	Assessment of Local Feed Resources for Enhancing Fertility and Productivity of Smallholder Dairy Cattle **Objective**: To assess the potential of local feed resources for enhancing the fertility and productivity of smallholder dairy cattle in the Nakuru District of Kenya.	N. Odongo / M. Shamsuddin
KEN/5/028	Applying Nuclear Based Techniques to Control Animal diseases **Objective**: To improve the capacity to diagnose and carry out surveillance of contagious 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.	H. Unger
KEN/5/033	Using an Integrated Approach towards Sustainable Livestock Health and Nutrition to Improve Their Production and Productivity for Enhanced Economic Development **Objective:** To use an integrated approach to manage both livestock health and nutrition in order to improve their production and productivity for enhanced economic development.	N. Odongo / A. Diallo
LES/5/002	Using Nuclear and Molecular Techniques for Improving Animal Productivity and Control of Transboundary Animal Diseases to Enhance Livestock Production and Health **Objective:** To improve livestock production and health	G.Viljoen
MAG/5/016	Applying Nuclear Techniques to Optimize Animal Production **Objective:** To increase animal production through the improvement of animal health and control reproduction in the Amoron'i Mania region.	M. Shamsuddin / N. Odongo / I. Naletoski
MAG/5/020	Improving Stockbreeding Productivity Through the Application of Nuclear and Related Techniques for Reducing Rural Poverty **Objective:** To contribute to reducing rural poverty by improving the productivity of stockbreeding.	M. Shamsuddin
MAR/5/021	Improving Smallholder Dairy Productivity through Better Nutrition by Using Locally Available Forage and Browse Species **Objective:** To contribute to the improvement of smallholder dairy productivity through better nutrition using locally available forage and browse species	N. Odongo
MLI/5/025	Improving National Capacities to Characterize Serotypes of Major Animal Diseases Using Molecular Biology Techniques for the Development of a National Disease Control Strategy **Objective:** The main objective is identification of the various serotypes of the foot and mouth disease virus. The project would help the elaboration of a national strategy for control of the disease by formulating vaccines which are currently imported from Botswana.	I. Naletoski
MLW/5/001	Strengthening the Essential Animal Health and Veterinary Infrastructure for Disease Control and Management Services in Urban and Rural Areas **Objective:** To develop capacity and strengthen infrastructure for animal disease control and management services in urban and rural areas of Malawi.	H. Unger
MON/5/020	Improving the Health Status of Livestock by Developing a Technology to Produce the Vaccine and Diagnostic Kit for Transboundary Animal Diseases **Objective:** To improve the health status of livestock by developing a technology to produce the vaccine and diagnostic kit of transboundary animal diseases.	G. Viljoen
MON/5/021	Improving the Productivity and Sustainability of Farms Using Nuclear Techniques in Combination with Molecular Marker Technology **Objective:** To improve the productivity and sustainability of livestock and crop integrated farms through utilization of high yield, disease resistant new wheat varieties and other cereal varieties developed by the combined application of nuclear and molecular marker	N. Odongo

TC Project	Description	Technical Officer(s)
MOZ/5/002	Promoting sustainable Animal Health, Reproduction and Productivity Through the Use of Nuclear and Related Techniques **Objective**: To obtain sustainable improvement in animal reproduction and breeding and animal health through the use of nuclear and nuclear related technologies.	G. Viljoen
MYA/5/022	Improving Animal Productivity through the Use of DNA-Based Technology and Artificial Insemination **Objective:** To improve livestock productivity through the selection of superior breeding stock and to improve capacity in the use of molecular and related technologies for raising the genetic quality of local and adapted livestock breeds.	M. Shamsuddin
NAM/5/011	Establishing Research and Diagnostic Capacity for the Effective Control of Animal Diseases in the Northern Communal Areas and Improving Veterinary Public Health Services **Objective:** To control transboundary and parasite-borne animal diseases in the Central and Northern Communal Areas (NCA) and to improve veterinary-public health	H. Unger
NEP/5/002	Improving Animal Productivity and Control of Transboundary Animal Diseases Using Nuclear and Molecular Techniques **Objective:** To improve livestock productivity for food security by integrated management of animal nutrition, reproduction and health.	I. Naletoski
RAF/5/057	Strengthening Capacities for the Diagnosis and Control of Transboundary Animal Diseases in Africa (AFRA) **Objective**: To strengthen the diagnostic capacity of national veterinary services to monitor and control major transboundary animal diseases, particularly foot and mouth disease, peste des petits ruminants and contagious bovine pleuropneumonia.	H. Unger / A. Diallo
RAS/5/060	Supporting Early Warning, Response and Control of Transboundary Animal Diseases Objective: To establish a regional/national network of laboratories and training centres on early diagnosis, response and control of transboundary animal diseases and eradication programmes for zoonotic diseases.	H. Unger
RAS/5/063	Improving the Reproductive and Productive Performance of Local Small Ruminants by Implementing Reliable Artificial Insemination Programmes **Objective**: To improve small ruminants productivity by implementing reliable artificial insemination programmes	M. Shamsuddin / M. Garcia
RER/5/016	Supporting Coordinated Control of Transboundary Animal Diseases with Socioeconomic Impact and that Affect Human Health **Objective**: To reduce transboundary disease incidence in livestock and livestock products in the Euro-Asian Region	I. Naletoski
RLA/5/049	Integrated Control of Fascioliasis in Latin America (in support of National Programmes	G. Viljoen / I. Naletoski
SIL/5/011	Controlling Economically Important Livestock Diseases **Objective**: To design epidemiological surveys and adopt appropriate rapid laboratory techniques for the diagnosis of PPR and NCD in small ruminants and local chickens.	H. Unger / I. Naletoski
SIL/5/013	Establishing a Dual-Purpose Cattle Development Project for the Sustainable Contribution to Food Security, Poverty Alleviation and Improved Livelihoods of Communities Raising Cattle **Objective:** Sustainable contribution to food security, poverty alleviation and improved livelihoods of communities raising cattle.	M. Shamsuddin
SRL/5/042	Applying Molecular Diagnostics to Zoonotic Diseases **Objective**: To enhance the long-term epidemic preparedness by developing competence in molecular diagnosis and surveillance of zoonotic infections.	Kashyap (NAHU) / H. Unger

TC Project	Description	Technical Officer(s)
UGA/5/030	Improving the Diagnostic Capacity in Animal Diseases (Phase II) **Objective**: To strengthen the diagnostic capacity of the National Animal Diseases Diagnostics and Epidemiology Laboratory in the detection of animal disease and food-borne pathogens including drug residues.	H. Unger / I. Naletoski
UGA/5/032	Improving Animal Production and Productivity through Advanced Animal Disease Control and Animal Production Measures **Objective:** To improve animal production and productivity through advanced animal disease control and animal production measures	H. Unger
URT/5/027	Improving Livestock Production and Productivity through Sustainable Application of Nuclear and Related Techniques **Objective:** The broad objective of this project is to improve livestock production and productivity in the United Republic of Tanzania through sustainable application of various nuclear and nuclear related techniques.	N. Odongo / M. Shamsuddin / M. Garcia
URU/5/026	Increasing the Profitability of Dairy Producers by Improving Reproduction Efficiency, Rational Sustainable Use of Genetic Resources **Objective**: To implement integrated management strategies to improve the profitability of medium size grazing dairy farms by means of (a) integrated nutritional strategies; (b) strategic reproductive interventions; and (c) marker-assisted selection.	M. Shamsuddin / N. Odongo
ZAI/5/021	Upgrading Laboratory Services for the Diagnosis of Animal Diseases and Building Capacity in Vaccine Production to Support the Sustainability of Food Security and Poverty Alleviation **Objective:** To support the sustainability of food security and poverty alleviation through animal diseases diagnosis and immunisation.	G. Viljoen / I. Naletoski
ZAM/5/028	Improving Productivity of Dairy Animals Maintained on Smallholder Farms through Selected Breeding and Effective Disease Diagnosis and Control Using Isotopic and Nuclear Techniques **Objective:** To improve productivity of dairy animals maintained on smallholder farms in rural areas through selected breeding, effective disease diagnosis and control, improved supply of quality feeds and application of assisted animal reproduction technologies.	N. Odongo / I. Naletoski / M. Shamsuddin / M. Garcia
ZIM/5/016	Strengthening Food Security and Safety by Advancing Technologies for the Rapid Diagnosis of Diseases of Major Economic and Zoonotic Importance and for Residue/Pesticide Control in Animals and Animal Products **Objective:** Strengthening the existing technology and capacity to rapidly diagnose diseases of major economic and zoonotic importance and enable proper and timely response to disease outbreaks.	I. Naletoski

Publications

Factors affecting the first service conception rate of cows in smallholder dairy farms in Bangladesh

M.A.R. Siddiqui, Z.C. Das, J. Bhattacharjee, M.M. Rahman, M.M. Islam, M.A. Haque, J.J. Parrish and M. Shamsuddin

Reproduction in Domestic Animals doi: 10.1111/rda.12114

The successful outcome of an insemination is a combination of both male and female fertility-linked factors. We investigated the first service conception rate of cows at artificial insemination (AI) in the smallholder dairy farms in Bangladesh. Frozen straws were prepared from ejaculates of Bos indicus (n = 7) and Bos indicus × Bos taurus (n = 7) AI bulls. Fertility was determined from 6101 first services in cows that were performed by 18 technicians in four regions between April 2004 and March 2005. Pregnancy was diagnosed by rectal palpation between 60 and 90 days post-insemination. The Asian version of Artificial Insemination Database Application (AIDA ASIA) was used for bulls-, cows- and AI-related data recording, and later retrieved for analysis. The mean ± SD number of inseminations performed from individual bulls and their conception rates were 436.0 ± 21.6 and 50.7 ± 1.9%, respectively. Logistic regression demonstrated body condition scores (BCS), heat detection signs, months of AI and their interactions had greatest effects (odds ratios: 1.24–16.65, $p < 0.04$–0.001) on first service conception rate in cows. Fertility differed ($p < 0.02$–0.001) between the regions, previous calving months, months of AI, BCS, parity and heat detection signs of cows. Inseminations based on mounting activity (n = 2352), genital discharge (n = 3263) and restlessness and/or other signs (n = 486) yielded a conception rate of 53.6%, 48.8% and 50.1%, respectively ($p < 0.05$). Conception rate between technicians ranged between 43.4% and 58.6% ($p < 0.05$). The days interval from calving to first service (overall mean ± SD = 153.4 ± 80.6) had relationship ($p < 0.001$) with BCS, months of previous calving and parity of the cows. Fertility at AI in smallholder farms can be improved by training farmers on nutrition and reproductive management of the cows.

Effect of exogenous enzymes and *Salix babylonica* extract or their combination on haematological parameters in growing lambs

Rivero, N., A.Z.M. Salem, H.M. Gado, M.G. Ronquillo, A.B. Pliego, C.G. Peñuelas and N.E. Odongo

Journal of Animal and Feed Sciences, 21: 2012, 577–587

The aim of this study was to compare the use of exogenous enzyme preparations (EZ) and/or *Salix babylonica* extract (SB) or their combination as feed additives on some haematological parameters in growing lambs. Twenty Suffolk lambs of 6 to 8-months-old with 24±0.3 kg body weight were used in the study. Lambs were divided into 4 groups of 5 animals each in a completely randomized design and the treatments were: 1.control: fed a basal diet of concentrate (30%) and maize silage (70%); 2. EZ: fed the basal diet plus 10 g of enzyme; 3. SB: fed the basal diet plus 30 ml of *S. babylonica* extract, and 4. EZSB: fed the basal diet plus 10 g enzyme and 30 ml of *S. babylonica* extract. Lambs were housed in individual cages and the experiment was conducted for 60 days. The SB was given orally while the EZ was mixed with a small amount of the concentrate and maize silage and was offered *ad libitum*. Blood samples were collected from each animal on days 0, 15, 30, 45 and 60 of experiment and analysed for haematological parameters. The treatments of EZ, SB or EZSB did not affect any of the measured blood parameters. Day of sampling modified concentrations of red blood cells (P=0.001; linear effect), haematocrit (P=0.01; quadratic effect), haemoglobin (P=0.01; linear effect), mean corpuscular volume (P=0.01; linear effect), monocytes (P=0.004; quadratic effect) and plasma protein (P=0.0002, linear effect). It could be concluded that *Salix babylonica* extract, exogenous enzymes and their combination as feed additives had not a negative effects on the blood parameters measured and therefore on the health of the lambs.

Reducing methane emissions and the methanogen population in the rumen of Tibetan sheep by dietary supplementation with coconut oil

Xuezhi Ding, Ruijun Long, Qian Zhang, Xiaodan Huang, Xusheng Guo, Jiandui Mi

Trop Anim Health Prod. 2012 Oct; 44(7):1541–5

The objective was to evaluate the effect of dietary coconut oil on methane (CH(4)) emissions and the microbial community in Tibetan sheep. Twelve animals were assigned to receive either a control diet (oaten hay) or a mixture diet containing concentrate (maize meal), in which coconut oil was supplemented at 12 g/day or not for a period of 4 weeks. CH(4) emissions were measured by using the 'tunnel' technique, and microbial communities were examined using quantitative real-time PCR. Daily CH(4) production for the control and forage-to-concentrate ratio of 6:4 was 17.8 and 15.3 g, respectively. Coconut oil was particularly effective at reducing CH(4) emissions from Tibetan sheep. The inclusion of coconut oil for the control decreased CH(4) production (in grams per day) by 61.2%. In addition, there was a positive correlation between the number of methanogens and the daily CH(4) production ($R = 0.95$, $P < 0.001$). Oaten hay diet containing maize meal (6:4) plus coconut oil supplemented at 12 g/day decreases the number of methanogens by 77% and a decreases in the ruminal fungal population (85–95%) and Fibrobacter succinogenes (50–98%) but an increase in Ruminococcus flavefaciens (25–70%). The results from our experiment suggest that adding coconut oil to the diet can reduce CH(4) emissions in Tibetan sheep and that these reductions persist for at least the 4-week feeding period.

Nutrient digestibility and ruminal fermentation characteristic in swamp buffaloes fed on chemically treated rice straw and urea

Nguyen VT, Wanapat M, Khejornsart P, Kongmun P.

Trop Anim Health Prod. 2012 Mar; 44(3):629–36

The experiment was conducted to determine effects of urea-lime-treated rice straw and urea levels in concentrate on rumen fermentation, apparent nutrient digestibility, and cellulolytic bacteria population of 4-year-old, rumen-fistulated swamp buffaloes. All animals were randomly assigned according to a 2×2 factorial arrangement in a 4×4 Latin square design to receive four dietary treatments: factor A, two sources of roughage (rice straw and 2%urea + 2%lime-treated rice straw); factor B, two levels of urea in concentrate mixture (0% and 4%). Roughages were given ad libitum together with 0.3% BW of concentrate. It was found that voluntary feed intake, the digestibility of DM, OM, CP, NDF, acetate, and propionate concentration were significantly increased ($P < 0.05$) by treated rice straw, while NH(3)-N, BUN, and propionic acid concentration were increased by both factors of treated rice straw and 4% urea in concentrate. The real-time PCR quantification of Fibrobacter succinogenes and Ruminococcus albus population, and anaerobic fungi were greater ($P < 0.05$), but the population of Ruminococcus flavefaciens, protozoa, and methanogenic bacteria were reduced ($P > 0.05$) as influenced by treated rice straw and urea level. In conclusion, the combined use of urea-lime-treated rice straw and fed with concentrate (4% urea) could improve rumen ecology, rumen fermentation efficiency, and nutrient digestibility in swamp buffaloes.

Methane mitigation from ruminants using tannins and saponins

Goel G, Makkar HP.

Trop Anim Health Prod. 2012 Apr; 44(4):729–39

The concentration window for tannins, at which in vivo anti-methanogenic effects without decreasing organic matter digestibility and productivity of animals have yet to be observed, is expected to be narrower than for saponins. Furthermore, for tannins, substantial decrease in methane reduction would be difficult to achieve without compromising production; however, simultaneous benefits that could be accrued, for example decrease in rumen protein degradability and increase in post-rumen protein availability, partitioning of excreted nitrogen more towards faeces and lesser towards urine, and increase in efficiency of microbial protein synthesis recorded in earlier studies (Makkar, 2003), might make the use of tannins attractive.

Among the tannin assays, tannin bioassay (a reflection of tannin activity) is the best predictor of the methane reduction potential of a plant. Total phenols and total tannins are also good predictors of the methane reduction potential. For screening a large number of tannin-containing plants and plant products, these assays could provide useful information on the potential candidates for further studies. In in vitro, methane decrease by addition of phenolic acids is relatively small, and the effect of phenolic acids on methane reduction depends on their concentration and number of hydroxyl groups on them. The higher the number of hydroxyl groups, the higher the potential methane reduction. Hydrolysable tannins appear to decrease methane production, and methane production per unit organic matter is digested to a greater extent than condensed tannins. The condensed tannins decrease methane more through reduction in fibre digestion (indirect effect), while hydrolysable tannins appear to act more through inhibition of the growth and/or activity of methanogens- and/or hydrogen-producing microbes (direct effect). In vitro, the saponin-containing plants did not produce substantial reduction in methane production but showed the potential to partition higher proportion of the substrate to microbial mass production. The saponins tested possessed anti-protozoal activity but did not al-

ways result in methane inhibition suggesting that the unidirectional relationship between protozoal numbers and methanogenesis, as affected by saponins, is not obligatory.

In vitro evaluation, in vivo quantification, and microbial diversity studies of nutritional strategies for reducing enteric methane production.

Abdalla AL, Louvandini H, Sallam SM, Bueno IC, Tsai SM, Figueira AV.

Trop Anim Health Prod. 2012 Jun; 44(5):953–64

The main objective of the present work was to study nutritive strategies for lessening the CH(4) formation associated to ruminant tropical diets. In vitro gas production technique was used for evaluating the effect of tannin-rich plants, essential oils, and biodiesel coproducts on CH(4) formation in three individual studies and a small chamber system to measure CH(4) released by sheep for in vivo studies was developed. Microbial rumen population diversity from in vitro assays was studied using qPCR. In vitro studies with tanniniferous plants, herbal plant essential oils derived from thyme, fennel, ginger, black seed, and Eucalyptus oil (EuO) added to the basal diet and cakes of oleaginous plants (cotton, palm, castor plant, turnip, and lupine), which were included in the basal diet to replace soybean meal, presented significant differences regarding fermentation gas production and CH(4) formation. In vivo assays were performed according to the results of the in vitro assays. *Mimosa caesalpineaefolia*, when supplemented to a basal diet (Tifton-85 hay *Cynodon* sp, corn grain, soybean meal, cotton seed meal, and mineral mixture) fed to adult Santa Ines sheep reduced enteric CH(4) emission but the supplementation of the basal diet with EuO did not affect ($P > 0.05$) methane released. Regarding the microbial studies of rumen population diversity using qPCR with DNA samples collected from the in vitro trials, the results showed shifts in microbial communities of the tannin-rich plants in relation to control plant. This research demonstrated that tannin-rich *M. caesepineapholia*, essential oil from eucalyptus, and biodiesel coproducts either in vitro or in vivo assays showed potential to mitigate CH(4) emission in ruminants. The microbial community study suggested that the reduction in CH(4) production may be attributed to a decrease in fermentable substrate rather than to a direct effect on methanogenesis.

Feeding of tropical trees and shrub foliages as a strategy to reduce ruminal methanogenesis: studies conducted in Cuba

Delgado DC, Galindo J, González R, González N, Scull I, Dihigo L, Cairo J, Aldama AI, Moreira O.

Trop Anim Health Prod. 2012 Jun; 44(5):1097–104

The aim of this paper was to present the main results obtained in Cuba on the effects of feeding tropical trees and shrubs on rumen methanogenesis in animals fed with low quality fibrous diets. More than 20 tree and shrub foliages were screened for phytochemicals and analyzed for chemical constituents. From these samples, seven promising plants (*Samanea saman, Albizia lebbeck, Tithonia diversifolia, Leucaena leucocephala, Trichantera gigantea, Sapindus saponaria*, and *Morus alba*) were evaluated for methane reduction using an in vitro rumen fermentation system. Results indicated that the inclusion levels of 25% of Sapindo, Morus, or Trichantera foliages in the foliages/grass mixtures (grass being *Pennisetum purpureum*) reduced ($P < 0.01$) methane production in vitro when compared to Pennisetum alone (17.0, 19.1, and 18.0 versus 26.2 mL CH(4)/g fermented dry matter, respectively). It was demonstrated that *S. saman, A. lebbeck*, or *T. diversifolia* accession 23 foliages when mixed at the rate of 30% in *Cynodon nlemfuensis* grass produced lower methane compared to the grass alone. Inclusion levels of 15% and 25% of a ruminal activator supplement containing 29% of *L. leucocehala* foliage meal reduced methane by 37% and 42% when compared to the treatment without supplementation. In vivo experiment with sheep showed that inclusion of 27% of *L. leucocephala* in the diet increased the DM intake but did not show significant difference in methane production compared to control diet without this foliage. The results of these experiments suggest that the feeding of tropical tree and shrub foliages could be an attractive strategy for reduction of ruminal methanogenesis from animals fed with low-quality forage diets and for improving their productivity.

Effects of plants containing secondary compounds and plant oils on rumen fermentation and ecology

Wanapat M, Kongmun P, Poungchompu O, Cherdthong A, Khejornsart P, Pilajun R, Kaenpakdee S.

Trop Anim Health Prod. 2012 Mar; 44(3):399–405

A number of experiments have been conducted to investigate effects of tropical plants containing condensed tannins and/or saponins present in tropical plants and some plant oils on rumen fermentation and ecology in ruminants. Based on both in vitro and in vivo trials, the results revealed important effects on rumen microorgan-

isms and fermentation including methane production. Incorporation and/or supplementation of these plants containing secondary metabolites have potential for improving rumen ecology and subsequently productivity in ruminants.

The role of nuclear technologies in the diagnosis and control of livestock diseases - a review

Gerrit J. Viljoen, Antony G. Luckins

Trop. Anim. Health Prod. 2012 Oct; 44(7): 1341–1366

Nuclear and nuclear-related technologies have played an important role in animal health, particularly in relation to disease diagnosis and characterization of pathogenic organisms. This review focuses primarily on how and where nuclear technologies, both non-isotopic and isotopic methods, have made their impact in the past and where it might be expected they could have an impact in the future. The review outlines the extensive use of radiation attenuation in attempts to create vaccines for a multiplicity of pathogenic organisms and how the technology is being reexamined in the light of recent advances in irradiation techniques and cryopreservation/lyophilization that might obviate some of the problems of maintenance of viable, attenuate vaccines and their transport and use in the field. This approach could be used for a number of parasitic diseases where vaccination has been problematic and where investigations into the development of molecular vaccines have still failed to deliver satisfactory candidates for generating protective immune responses. Irradiation of antigens or serum samples also has its uses in diagnosis, especially when the samples need to be transported across international boundaries, or when handling the pathogens in question when carrying out a test presents serious health hazards to laboratory personnel. The present-day extensive use of enzyme immunoassays and molecular methods (e.g., polymerase chain reaction) for diagnosis and characterization of animal pathogens has its origins in the use of isotope-labeled antigens and antibodies. These isotopic techniques that included the use of ^{75}Se, ^{32}P, ^{125}I, and ^{35}S isotopes enabled a level of sensitivity and specificity that was hitherto unrealized, and it is prescient to remind ourselves of just how successful these technologies were, in spite of their infrequent use nowadays. Finally, the review looks at the potential for stable isotope analysis for a variety of applications—in the tracking of animal migrations, where the migrant are potential carriers of transboundary animal diseases, and where it would be useful to determine the origins of the carrier, e.g., highly pathogenic avian influenza and its dissemination by wild water fowl. Other applications could be in monitoring sequestered microbial culture (e.g., rinderpest virus) where in the case of accidental or deliberate release of infective culture it would be possible to identify the laboratory from which the isolate originated.

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CD-ROMs

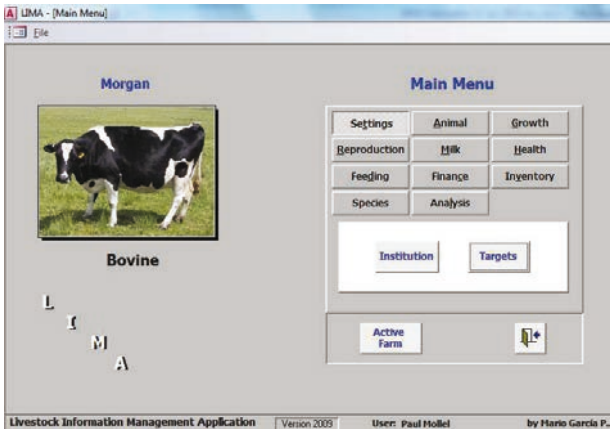
A CD-ROM is available dealing with training material for the diagnosis of rinderpest and for the preparation for the OIE pathway. It was produced under an IAEA Technical Cooperation project RAF/0/013 ICT based training to strengthen LDC capacity. Contact Gerrit Viljoen at g.j.viljoen@iaea.org for further information.

Database applications

Four computer database applications to monitor livestock reproductive performance can be downloaded *software and manuals) from the IAEA ftp server ftp://ftp.iaea.org/pub/NAFA/APH/Mario/Databases/ These applications were developed through the implementation of various Regional TC projects and have been updated several times, especially LIMA and SPeRM thanks to the suggestions and recommendations of the database users. All are available in English for downloading but LIMA can also be available in Spanish. French versions for LIMA and SPeRM are under preparation.

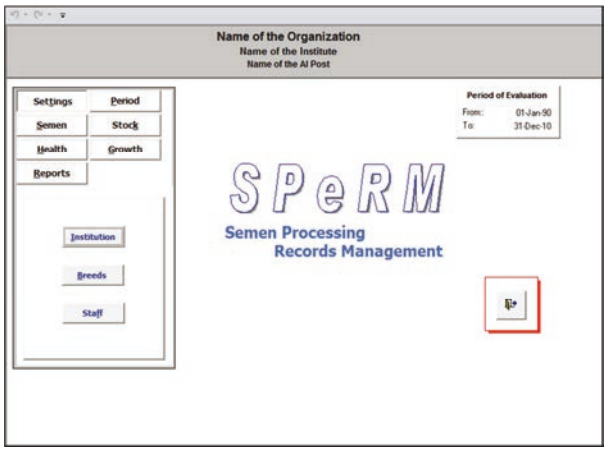
It is recommended to contact Mario Garcia (M.Garcia-Podesta@iaea.org; mggarciap@gmail.com) before installing the application for advice.

Livestock Information Management Application (LIMA)



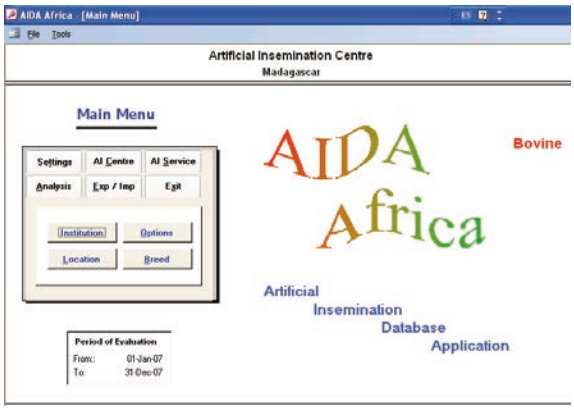
LIMA is a computer application to store and analyse a full range of information from livestock farms. LIMA is suitable for six livestock species, i.e. bovine, bubaline, ovine, caprine, and South American camelids (alpacas and llamas) and is available in English and Spanish. The application contains convenient and easy-to-use data entry forms for the identification of the animal, productive records (body weight, milk yield, wool and fibre production), reproductive parameters (heats, services, parturitions), health data (individual cases and collective preventive treatment), and economical information (farm income and expenses). Moreover, there is a wide collection of predefined reports for the analysis of the data, and facilities for data verification and export.

Semen Processing Records Management (SPeRM)



SPeRM is a computer application to store and analyse information from sires (bulls, bucks, and rams) that are used in Semen Processing or Artificial Insemination (AI) Centres.

Artificial Insemination Database Application (AIDA Asia) / (AIDA Africa)



AIDA-Asia and AIDA-Africa are computer applications to store and analyse information from AI Services (farms, females inseminated, semen, estrus characteristics, inseminator and pregnancy diagnosis data). Field data can be complemented with progesterone radioimmunosasay data from milk or blodd samples collected at four key times during artificial insemination service and the oestrous cycle. Both applications are very similar; however, AIDA-Africa has two levels of data entry as compared to three levels for AIDA-Asia, due to the more complex structure of AI in most Asian countries.

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