• Established laboratories for proximate feed assays and basic expertise on conducting animal nutrition experiments.

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

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://cra.iaea.org/cra/index.html

General information applicable to all coordinated research projects and submission of proposals:

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/.

Activities of the Animal Production and Health Laboratory

Animal Genetics

Genetic variation on the control of resistance to internal parasites in small ruminants for improving animal productivity

Large scale genotyping of sheep and goat under field trial for parasite resistance

Gastro-intestinal (GI) parasitic infestations incur huge economic losses to poor and marginal farmers rearing sheep and goats across the world. The loss per year has been estimated to the tune of $10 billion. Breeding programs with the goal of enhancing host resistance to parasites will help to alleviate this problem in the long term. In continuation of its efforts to establish a low density DNA marker panel for parasite resistance, Animal Production and Health Laboratory (APHL) identified and developed genotyping assays for novel candidate gene SNP (single nucleotide polymorphism) markers to be associated with phenotypes of Haemonchus resistance in sheep and goats. Large scale genotyping of 1367 goat samples derived from field trials performed in Bangladesh, China, Nigeria, Sri Lanka, Pakistan, Myanmar and India were completed. A panel of 141 goat SNP markers located in 72 candidate genes including pattern recognition receptors (Toll like receptors, NOD like receptors, RIG I like receptors, C type Lectin binding receptors), cytokine genes (e.g. Interleukins, Interferons), Caprine histocompatibility genes was used to type 18 indigenous goat breeds for evaluation of parasite resistance. In case of sheep, 1524 samples derived from field trials performed in Argentina, Brazil, Ethiopia, Iran, Indonesia and India were completed. A panel of 174 sheep SNP markers was used to type 10 indigenous sheep breeds for evaluation of parasite resistance. Datasets of genotypes from a total of 3115 sheep and 1367 goats have been generated and statistical analysis is currently under progress in collaboration with counterparts from various member states.

Genome wide association study (GWAS) for parasite resistance in sheep

In addition to candidate gene polymorphisms, genetic variations located throughout the genome play a significant role in the inheritance of traits related to parasite resistance. Hence, genome wide analysis of Corriedale sheep from Argentina exhibiting extreme phenotypes was initiated. 48 sheep with low EBVs (Estimated Breeding Values) for faecal egg count (supposed to be relatively resistant/tolerant sheep) and 48 sheep with high EBVs for faecal egg count (supposed to be relatively susceptible sheep) were completed using a 60K Affymetrix microarray. Genotyping will be extended to additional sheep samples with phenotype extremes and to other breeds to perform a genome wide association study and identification of genomic regions under selection for parasite resistance.

Support to MSs for implementation of Global Plan of Action on animal genetic resources (AnGR)

Genetic characterization of indigenous buffalo populations of Myanmar
Water buffalo (*Bubalus bubalis*) is an important livestock in South and South East Asia. Buffaloes are valuable not only as milk producers, but have multiple roles in rural livelihood system, particularly as a draught animal in paddy cultivating areas and contribute significantly to employment generation and nutritional security. Myanmar has a long history of raising buffaloes for draught purposes with an estimated population of 2.6 million. There are 2 types of buffaloes in Myanmar, one is smaller, commonly found in low lands and the other is bigger and found in the hilly regions like Shan State. They have more working capacity than oxen and are well suited to work in low-lying swampy areas. Although most of these buffaloes are considered to be swamp type, river buffaloes are also available in some areas. Characterization and documentation of these buffalo populations is of prime significance for genetic improvement and biodiversity conservation programs. APHL initiated and completed the design, development and optimization of DNA marker panels for genetic characterization of water buffaloes. Six multiplex panels covering 21 microsatellite DNA markers were standardized for genotyping and the marker panels performed well for characterization of both the sub-species of buffaloes, river and swamp types. Indigenous buffalo populations from three different provinces of Myanmar were genotyped and sequenced (mitochondrial DNA D-loop variations). Statistical analysis of genotype and sequence data is currently under progress.

**Mapping molecular diversity of indigenous goat genetic resources of Asia**

The world goat population is approximately 1.0 billion with more than half of them present in Asia. The Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture initiated a programme to characterize goat genetic resources of Asia. Nine Asian countries, Bangladesh, China, Indonesia, Iran, Pakistan, Sri Lanka, Vietnam, Myanmar and India, were supported to conduct breed surveys, evaluate production environments and assess phenotypic and genetic characteristics of indigenous breeds/populations. A meta-analysis of genotypes from 2249 goats belonging to 57 goat breeds located in 9 Asian countries was conducted to assess genetic diversity, relationship and population structure. The level of genetic variability among goat breeds/populations across Asia was consistent with the history of domestication, with variability being higher near the center of domestication and a decreasing gradient while moving away from this center.

Genetic differentiation among goat breeds/populations within different countries varied from 1.9% (Myanmar goats) to 12.6% (Indonesian goats) with a global $F_{ST}$ of 12.7% (Figure 1). Genetic differentiation among local goats within countries was limited, an indication of high gene flow across breed/populations.

![Figure 1. Between (red) and within (yellow) breed variation; mean percent loci deviating from Hardy-Weinberg equilibrium (Dark red: Significant heterozygosity deficit ($P<0.05$); Orange—Significant heterozygosity excess ($P<0.05$); Green—Not deviating from Hardy-Weinberg equilibrium ($P>0.05$)) among indigenous goat populations from Asian countries.](image)

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**Application of irradiation technology to develop a potential trypanosome vaccine**

Trypanosomosis, a parasite disease in mammals, remains a big hindrance to the development of livestock resources in Africa. The disease puts a large number of cattle at risk with annual losses estimated to be as high as US$5 billion. A vaccine would provide the most effective means of managing the disease in Africa and other endemic areas. At APHL, experiments have been carried out to characterize the effects of using low level irradiation doses on trypanosomes. These experiments showed that immunisation with low dose irradiated parasites induce a stronger immune response when compared to high dose irradiated parasites. To further study the effect of low dose irradiation on protozoan parasites, an expression micro-
array platform that covers the genomes of three trypanosome species, *T. brucei*, *T. evansi* and *T. congolense* was designed at APHL, so as to give a global view of irradiation effect on gene expression (Figure 2).

Expression analysis of *T. evansi* genome irradiated using doses ranging from 0 Gy to 250 Gy was carried out using the Affimetrix platform. At least six technical replicates per irradiation dose were used for expression analysis and initial results indicate that at least 3534 genes (p > 0.05) are differentially expressed when subjected to different irradiation doses. These results are now being analysed in depth to identify genes responsible for loss of virulence and infectivity. The classification of differentially expressed genes according to function i.e. are the genes affected by irradiation responsible for structural, metabolic or enzymatic processes will be important in deciding which targets to develop for new vaccines and drugs.

**African swine fever**

African swine fever (ASF) is one of the most devastating transboundary animal diseases for the swine industry, both in Africa and the Balkan region. The disease also represents a serious threat to Europe with the recent detection of ASF in wild boar in several countries (including Russia, Belarus, Lithuania, Poland, and more recently Latvia, Estonia and Italy), showing the continued movement of virus within Europe. APHL continued to collaborate with MSs to assess the epidemiology of ASF virus (ASFV) and study the viral genome through IAEA technical cooperation projects, coordinated research projects (CRP D3.20.31- Early and Rapid Diagnosis and Control of Transboundary Animal Diseases — Phase II: African Swine Fever) and extra budgetary projects (Peaceful Uses Initiative). Molecular characterization of ASFV in Ethiopia, Mozambique, Nigeria, Tanzania, and Mali showed new genetic variants emphasizing the need for continued monitoring and characterization of circulating ASFV strains to be able to provide information that could lead to the production of a successful vaccine. APHL also continued to assist MSs in the detection of ASF particularly in Africa. Recently, real-time PCR based ASF diagnostic technology was transferred to Ghana and Mozambique to improve the capacities of national veterinary laboratories in handling suspected outbreaks.

**Study of pox diseases in Ethiopian camels**

Camels are economically important animals that are well adapted to arid and semi-arid climates and are valued for nomadic pastoralism for transportation, racing, and production of milk, wool and meat. Two major pox diseases are known in camels: camelpox and camel contagious ecthyma. Camelpox is an infectious disease caused by camelpox virus (CMPV) and belongs to the genus Orthopoxvirus of the family Poxviridae. Camel contagious ecthyma, also known as Auzdyk disease, is caused by pseudocowpox virus (PCPV) classified under the genus Parapoxvirus of the Poxviridae family.

As part of its work to improve the management of pox diseases in ruminants and camels, APHL has developed a pan-pox real time PCR method and started the transfer of the assay to Member states (MS). The current assay was used in Ethiopia as a front-line assay to investigate pox diseases in camels. This allowed the clear identification of CMPV and PCPV as two major causes of skin diseases of camels in the country. To further analyze and understand the epidemiology of these diseases in the country, representative samples, collected from diseased camels from different geographical locations of Ethiopia between 2011 and 2014, were molecularly analyzed. The full hemagglutinin gene for CMPV (HA, 925bp) and major envelope protein gene (B2L, 1137bp) for PCPV, of Ethiopian isolates were amplified, sequenced and compared to publicly available sequences. The results confirmed the circulation of CMPV and PCPV among one-humped camels (Camelus dromedarius).

**Figure 3. Contagious ecthyma in young camels. Note the presence of severe nodular lesions on the upper and lower lips and around nostril.**
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The nucleotide sequences of the B2L gene (1137nt) were used for 20 Ethiopian outbreak isolates and 13 sequences retrieved from GenBank.

Twenty-seven CMPVs and 20 PCPVs were identified from pox disease outbreaks samples and compared to foreign isolates. Three major clusters of PCPV were found in Ethiopia, with cluster 1 isolates closely related, but not identical to Sudanese PCPVs. They were more distant to all other PCPVs retrieved from cattle worldwide. For CMPV, all Ethiopian isolates formed a single cluster. They were closely related to CMPVs from Somalia and Syria. This study has also highlighted the existence of co-infection with CMPV and CPCPV in two samples collected in suspicion of camelpox and demonstrated the misdiagnosis of camelpox infections due to the similarity of the clinical symptoms to those of PCPV infections.

**Fellows/interns/consultants**

**Mr Lwin Ko Ko Kyaw** from Livestock Breeding and Veterinary Department, Yangon, Myanmar was trained on “Genetic characterization of indigenous buffaloes of Myanmar” at APHL for three months (6th July, 2015 to 2nd October, 2015) under TC fellowship (MYA/14013).

**Ms Brenda Chileshe** from National Institute for Scientific and Industrial Research, Lusaka, Zambia was trained on “Analysis of data for genetic characterization of Zambian native Zebu cattle using nuclear and extra-nuclear DNA markers” at APHL for three months (1st October, 2015 to 24th December, 2015) under TC fellowship (ZAM/14015).

**Mr Lourenço Paulo Mapaco** from the Central Veterinary Laboratory, Directorate of Animal Science, Mozambique was trained at APHL on laboratory diagnosis of transboundary animal diseases using molecular techniques for three months from 15th June to 14th September 2015 under IAEA/TC Project MOZ/5/005.

**Field support missions**

1. **Follow up report of a visit to the Laboratoire Central Vétérinaire de Bingerville, Abidjan, Cote d'Ivoire, by a APHL staff**

A staff member of the Animal Production and Health Laboratory (APHL) travelled from 25 to 29 May, 2015 to Abidjan, Cote d’Ivoire to transfer animal pathogen typing technologies to the Laboratoire Central Vétérinaire de Bingerville. The aims of this mission were (1) to transfer and implement technologies and build capacities, (2) to respond to the emergency H5N1 situation in Cote d’Ivoire and (3) to provide an emergency “tool kit”. During the week, LCV Bingerville was assisted in the set-up of their instrument and staff was trained on animal pathogen detection by real time nucleic acid amplification technology including multiparametric pathogen detection assays. Additionally, they were trained to use sequencing technology for a better identification and characterization of animal pathogens.

To date, the laboratory has provided valuable feedback on the successful adoption of the rapid detection and subtyping of H5N1 using a duplex real time PCR detection procedure. This technology is now being used as a front-line tool by the virology laboratory for the screening of suspected H5N1 cases in support of the national effort to control the current HPAI crisis in the country. Twenty seven (27) outbreaks were confirmed using this method in 5 geographical locations in the country: Bouaké (3 backyard poultry farms, 1 turtle dove bird), Abidjan (15...
live birds markets, 2 commercial farms and 3 backyard poultry farms), Grand-Bassam (1 commercial farm), Bingerville (1 live birds market, 1 backyard poultry farm) and Anyama (1 live birds market). All these confirmed outbreaks were newly affected areas.

Interestingly, there was a full agreement between the laboratory results and those provided by the OIE reference laboratory, Padova, Italy, which was requested to confirm the outbreaks. Moreover, the laboratory was also able to use sequencing technology to fully characterize the samples from one outbreak. This achievement highlights the capability of the virology laboratory of the LCV, Bingerville and the importance of the capacity building strategies adopted by the Joint FAO/IAEA division. It is of great importance to note that prior to the adoption of real-time PCR, the virology laboratory of LCV carried out the conventional RT-PCR to detect and fully subtype H5N1 isolates. The successful adoption of a closed vessel system for the rapid subtyping of H5N1 will facilitate the management of the current avian influenza crisis; laboratories will gain in speed and accuracy resulting in a faster delivery of results, while minimizing the risk of contamination, allowing for a quick response to newly emerging outbreaks. To this end, the current assay was also included as a component of the emergency tool box supplied by the joint division to many Western African veterinary laboratories to facilitate the identification of influenza A viruses of the H5N1 subtype.

2. Laboratoire Central de l’Elevage, Niamey, Niger

In January 2015, Nigeria confirmed the presence of HPAI H5N1 to the World Organisation for Animal Health (OIE) while neighbouring Burkina Faso and Niger reported outbreaks on 1st and 21st of April, 2015, respectively. A field support mission was made to Laboratoire central de l’élevage (LABOCEL), Niamey, Niger (25-28, August) along with the provision of an Emergency Tool Box containing the necessary reagents and material to immediately screen more that 200 samples for H5N1. During the four day mission, the personnel of LABOCEL were trained on a number of protocols through demonstration and practical sessions. These included the isolation and purification of RNA from swab samples, conventional RT-PCR identifying the H5 gene in clinical samples, conventional RT-PCR identifying the N1 gene in clinical samples and a duplex Real-Time PCR that simultaneously identifies the presence of the N1 and H5 genes in clinical samples. At the end of the mission, LABOCEL was able to implement all the assays for rapid diagnosis of H5N1 and acquired the capacity to screen large number of suspected samples.

3. Accra Veterinary Laboratory, Veterinary Services Directorate, Ministry of Food and Agriculture (MOFA), Accra, Ghana

The mission was carried out from 17 to 22 August 2015 to support the national and regional efforts to combat current HPAI H5N1 outbreaks in western Africa. An expert from APHL undertook this emergency travel to assist Ghana in the rapid identification of H5N1 cases currently occurring in Ghana and the potential spread from neighbouring countries such as Burkina Faso, Cote d’Ivoire, and Nigeria. The objective of the mission was to introduce real time PCR technology for the diagnosis of HPAI H5N1. A duplex H5N1 detection assay that detects both H5 HA and N1 NA in the same assay was transferred, thus greatly reducing the work time involved in detection and reporting. The laboratory staffs were quick to learn the concepts behind real time PCR and were sufficiently capable of running and analysing the data. Follow up with the laboratory director, Dr Joseph Awuni, has shown the continued use of this assay in rapidly and accurately detecting H5N1 in outbreaks in Ghana, with data reports being sent on a regular basis to IAEA.

4. Laboratoire National d’Elevage et de Recherches Vétérinaires, Dakar, Senegal

An expert from APHL travelled to Dakar, Senegal from 28 September to 2 October 2015. The main goals of this mission were to transfer new technology and coordinate future collaboration efforts between the laboratory staff and the joint FAO/IAEA division regarding African swine fever. This laboratory has been very capable of performing real-time PCR assays, so the focus was to transfer the working knowledge of several new multiplex assays that have been developed at IAEA. These real-time assays are based on multiple pathogen detection, whereby, one animal sample can be tested for many pathogens in a single procedure. The first assay involved detection of Pasteurella, Mycoplasma capricolum subesp. capripneumoniae (MCCP), peste des petits ruminants virus (PPRV) and capripoxvirus, from small ruminant animals. This assay is valuable because these pathogens can cause similar clinical symptoms that could delay treatment if the wrong pathogen is suspected. Another assay was a pan-poxvirus detection procedure that included testing of eight pathogens that can present with common clinical signs. This assay can differentiate between three genus, Orthopoxvirus, capripoxvirus, and Parapox virus. Within each genus, the assay can differentiate between CPXV, CMPV, SPVV, GTPV, LSDV, ORFV, PCPV, and BPSV. Transfer was also done with the duplex HPAI H5N1 real-time RT-PCR assay for avian influenza. While Senegal is currently not affected by HPAI, the mission helped to build capacity and prepare the laboratory for early and rapid diagnosis in case of cross border movement of disease. Laboratoire National d’Elevage et de Recherches Vétérinaires, in Dakar, Senegal.
5. The Central Veterinary Laboratory, Maputo, Mozambique

This mission was undertaken from 5th to 9th October 2015 by an expert from APHL, as part of the project to strengthen animal disease diagnostic capacities in selected Sub-Saharan African countries, supported by the South African Renaissance Fund (ARF). The mission included the transport and installation of a new Real-Time PCR platform (CFX96 Biorad) in the laboratory. A number of molecular diagnostic protocols were successfully demonstrated on the instrument for the detection of important transboundary animal diseases such as African swine fever, avian influenza and peste des petits ruminants. The laboratory staff were trained on the protocol for the identification and pathotyping of NDV. Field samples collected from different outbreaks in Mozambique between August and November 2014 were tested using the newly implemented protocol and the results were confirmed by conventional RT-PCR. The provision of a real-time instrument and the implementation of a new diagnostic protocol for NDV has provided an important tool to CVL for the diagnosis and pathotyping of NDV. Previously, using conventional diagnostic techniques the laboratory was only able to provide confirmatory diagnosis of NDV and was unable to determine whether the circulating viruses were velogenic, mesogenic or lentogenic, a vital piece of information for the correct control and management of this important disease.

6. Laboratoire Central Vétérinaire, Bamako Mali

APHL staff travelled from 31st August to 04th September to Bamako, Mali, for transferring animal pathogen typing technologies to the Laboratoire Central Vétérinaire (LCV), Bamako, Mali. The mission was carried out under the framework of the project to strengthen animal disease diagnostic capacities in selected Sub-Saharan African countries, supported by the South African Renaissance Fund (ARF), and Peaceful Uses Initiative (PUI) projects supported by USA and Japan. LCV recently received the molecular diagnostic platform under the project and the mission was undertaken to transfer real time PCR technology, focusing on multi-targets detection, as an additional tool for a more rapid and accurate diagnosis of transboundary animal diseases. The laboratory staff was trained on well-established protocols, including those developed at APHL, as well as protocol selection procedures to facilitate the implementation of new assays by the laboratory. 15 scientists and technicians of all departments of the LCV participated in the training (Figure 6). The primary objectives of the training were successfully achieved as the laboratory staff was able to set up, execute and interpret the results of real time PCR assays for the detection of African swine fever, peste des petits ruminants viruses and CaPV genotyping.

![Figure 6. A scientist of LCV, Bamako, Mali, operating the real time PCR instrument](image-url)

They were also able to perform and analyze the results of multi-parametric assays to detect pathogens responsible for respiratory diseases and pox-like lesions in ruminants. Given the recent emergency of HPAI H5N1 outbreaks in several Western African countries, the mission also ensured LCV to acquire capacity for H5N1 diagnosis. An emergency "tool kit" of supplies for H5N1 diagnosis was provided along with the required training. It is expected that this technology will help the laboratory to better fulfil its mandate within the national strategies for the control of transboundary animal diseases.