Joint FAO/IAEA Programme
Nuclear Techniques in Food and Agriculture

Animal Production & Health Newsletter

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To Our Readers

Scientists from Animal Health Institute and National Veterinary Institute, Ethiopia, being trained at the Animal Production and Health Laboratories, September 2023

Dear colleagues,

I would like to start by introducing myself as the new Section Head of the Animal Production and Health Sub-programme of the Joint FAO/IAEA Centre, who joined on 1 September 2023. I take this opportunity to thank all staff of the Joint FAO/IAEA Centre for such a warm welcome and introduction to a vibrant and self-driven team that thrives to improve animal production and health using nuclear and related technologies with the goal of improving food security and livelihoods amongst Member States (MSs). I also take this opportunity to thank and acknowledge my predecessor, Mr Gerrit Viljoen, who served the IAEA for 20 years and retired from the Agency on 31 May 2023. I also thank and acknowledge Mr Giovanni Cattoli, the former Head of the Animal Production and Health Laboratory, who left the Agency on 31 August 2023 after having served for almost 8 years. Together they tirelessly and successfully built a great team and relevant programmes that seek to address the current challenges of food and agriculture through nuclear and related technologies' facilitated improvement in animal production and health.

As part of its Coordinated Research Programme and Technical Cooperation Programme, the Animal Production and Health Section (APH), through its laboratory at Seibersdorf (APHL), continuously seek to identify, establish and disseminate current and relevant nuclear and related technologies to address the challenges affecting animal agriculture with a major focus on (i) early and rapid detection, characterisation, surveillance, monitoring and control of animal and zoonotic disease (ii) genetic characterisation and improvement of animal genetic resources for improved productivity, resilience to diseases, climate change and animal welfare, and (iii) improvement in animal nutrition and reproduction amid challenges of limited resources and climate change.

This second and final half of the year witnessed a number of research and development activities through the coordinated research projects (CRPs) and capacity building in MSs through the technical cooperation projects (TCPs). Nine contracts were awarded for the newly launched CRP on innovative nuclear and related molecular approaches for detection and characterization of antimicrobial resistance

(AMR) in animal production environment. The CRP will help to develop validated/harmonized protocols for sampling and analysis of farm environmental samples, investigate the distribution and characteristics of drug resistance among infectious agents affecting livestock. Additionally, the CRP will produce scientific data on performance of candidate alternative substances to animal growth promoters in animal production including strategies/guidelines on optimal husbandry practices that improve biosecurity and mitigate AMR in animal farm premises. Considering the significance of beta casein, milk protein variants (A1 milk vs A2 milk) in human health, there has been growing interest in several FAO/IAEA MSs to identify and breed cows that produce A2 milk that is easy to digest and associated with less discomforts. APHL has in this year, developed, and validated a simple, cost-effective DNA test that can help farmers and breeders to differentiate cows that produce A1 and A2 milk.

With respect to implementation of Global Action Plan on Animal Genetic Resources in FAO/IAEA MSs, APH supported the study on genomic characterization of indigenous Myanmar goat breeds and their relationship with sheep breeds in neighbouring South Asian countries. APH supported Cambodia, Madagascar and Senegal in improving the capacities of national artificial insemination networks through strengthening laboratories for frozen semen production. APH also supported eight Latin American countries on the application of genomic technologies to improve breeding for enhanced host resistance against infectious diseases in aquaculture. Several other CRPs focusing on animal nutrition and breed genetic characterisation and improvement are ongoing and the progress made described in detail in this newsletter.

The ongoing IAEA flagship ZODIAC initiative and Veterinary Diagnostic Laboratory (VETLAB) Network programme continue with their efforts to develop and disseminate tools for early and rapid detection, characterisation and surveillance of animal and zoonotic diseases, build networks amongst MSs and support them to be better prepared to tackle disease outbreaks and pandemics. This becomes critical in the aftermath of the COVID-19 pandemic and as we see continued and increased outbreaks and spread of diseases such as African swine fever (ASF) and highly pathogenic avian influenza (HPAI) H5N1 and as we together with other organisations and agencies work towards eradication of peste-des-petite-ruminants (PPR) by 2030. The 7th coordination meeting of the VETLAB Network took place at the IAEA headquarters in Vienna from 21 to 25 August 2023. The annual gathering included directors and focal points from African and Asian national veterinary laboratories, international organizations (e.g., FAO, WOAH), FAO reference centres, and research institutions In addition, APHL supported VETLAB partner laboratories to characterise local isolates of lumpy skin disease virus (LSDV) in Indonesia, Lesotho, Libya, Sri Lanka and Tanzania and ASF in Mozambique and Mali as well as SARS-CoV-2 in beavers in Mongolia. The DIVA ELISA for detection of antibodies against the Capripox virus, developed at APHL will soon be disseminated through a peer reviewed publication that is in press and further on through the TC programme to MSs veterinary laboratories. Considering the successes in the African and the Asian regions, APH is looking forward to expanding the VETLAB Network to Europe and Latin America, so that the veterinary laboratories in that regions can benefit from the deliverables of the VETLAB Network. Extrabudgetary funding is required to support this expansion.

The first coordinated research meeting for the ZODIAC CRP in Asia was held from 11 to 14 December 2023 in the Republic of Korea. The aim of this CRP is to enhance laboratory preparedness for the detection and control of emerging and re-emerging zoonotic diseases in Asia and the Pacific.

A number of training courses were held at the APHL and in various MSs and these included (i) a training course on multiparametric detection of pathogens causing major transboundary animal diseases and zoonoses held from 25 September to 6 October 2023 at APHL Laboratory in Seibersdorf, Austria; (ii) training course on detection and differential diagnosis of PPR and other small ruminants respiratory diseases held from 23 October to 3 November 2023 at the Institute of Veterinary Research (IRVT), Tunis, Tunisia; (iii) training course for VETLAB Network partners on next generation sequencing, bioinformatics and molecular phylogeny held from 20 November to 1 December 2023 at APHL Laboratory in Seibersdorf, Austria; and (iv) a national training course on bioinformatics analysis of data for genomic characterization of livestock that was hosted at Laboratoire National de L'Elevage et de Rechereches Veterinaires, Institut Seenegalais de Recherches Agricoles, Dakar, Senegal from 13 to 24 November 2023; amongst numerous other training courses and workshops detailed in this newsletter.

At the APH laboratory in Seibersdorf, innovative strategies for generating permissive cell lines capable of producing a greater quantity of viral particles via CRISPR/Cas9, a gene-editing technology, are being explored which will facilitate production of less expensive inactivated viral vaccines against zoonotic and livestock diseases. As APH-led irradiated vaccine research advances from the laboratory to the field, these strategies will prove beneficial in the commercialization of such vaccine candidates.

On 18 October 2023, the IAEA and FAO jointly launched the Atoms4Food initiative. The Atoms4Food Initiative seeks to provide Member States with ground-breaking solutions tailored to their specific needs and circumstances, by harnessing the advantages of nuclear techniques along with other advanced technologies, to enhance their innovation capacity so as to boost food and nutrition security, while keeping sustainable natural resource management. The APH

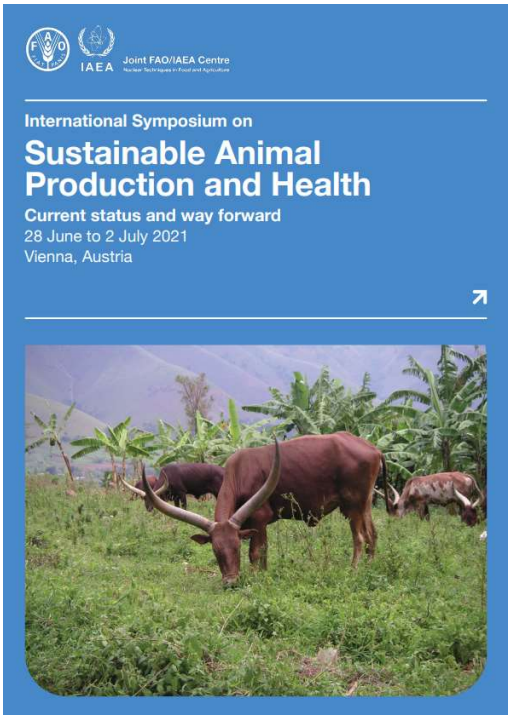
will, through this initiative, contribute to improved and sustainable food production through improved reproduction and breeding and control of animal and zoonotic diseases. R&D, support and capacity building for the application of nuclear and related technologies for sustainable animal production and improvement in MSs will be used to achieve these goals.

I would like to end by welcoming Ms Elena Ahasic who joined APH during this period and I acknowledge her valuable expertise and the additional capacity she is providing towards APH programmes.

Farai Muchadeyi

Head, Animal Production and Health Section

The International Symposium on Sustainable Animal Production and Health – Current Status and Way Forward - Book of Synopses Published



The International Symposium on Sustainable Animal Production and Health – Current Status and Way Forward, organized by the Animal Production and Health Section of the Joint FAO/IAEA Centre of Nuclear Techniques in Food and Agriculture, found its departing point in these challenges. During the five days of discussions and debates, the Symposium consisted of a panel discussion and eight thematic sessions: a) molecular tools for animal production and health, b) advances in vaccinology, c) emergency preparedness and response, d) zoonotic diseases, COVID-19 and ZODIAC, e) enhancing livestock's contribution to One Health and the Sustainable Development Goals, f) challenges for better livestock production in the developing world; g) advances in biotechnologies for improving livestock breeding and feeding, h) application of improved technologies for sustainable livestock productivity: the way forward.

The symposium, held virtually, was attended by more than 3000 participants and observers from more than 160 countries, as well as by representatives of international organizations including the International Atomic Energy Agency (IAEA), the Food and Agriculture Organization of the United Nations (FAO) and the World Organization for Animal Health (OIE). The more than 50 presentations were related to research and development actions for the sustainable improvement of animal production and health, emphasizing the role of nuclear technologies. These presentations were complemented by more than 145 synopses and posters from the participants, which were made available in a Book of Synopses.

This publication is a compilation of the contributions emanating from the symposium. It encompasses the three opening speeches of the IAEA Director General, Mr. Rafael Mariano Grossi, the FAO Director General, Mr. Qu Dongyu, and the OIE Director General, Ms. Monique Eloit; and 47 papers from participants and speakers, which have been peer-reviewed by FAO and IAEA colleagues, independent external experts and the Scientific Committee. The Book of Proceedings provides vital information and evidence on how nuclear and nuclear related techniques can contribute to the development of sustainable livestock production systems, as well as noting the constraints and opportunities for their use in developing countries. The book hopes to serve as guidance for scientists as well as government and institutional policy and decision makers.

Click here for more information

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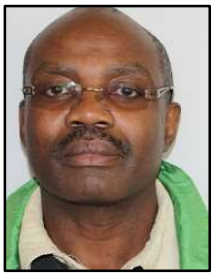
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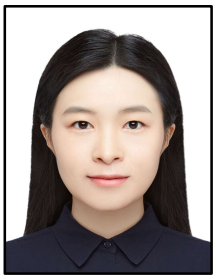
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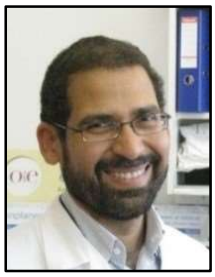
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VETLAB Network Bulletin

01/2024

In this issue:

VETLAB is an initiative of the Joint FAO/IAEA Centre

To the readers

The annual meeting of the Directors of the Veterinary Laboratories partnering the VETLAB Network was hosted at the IAEA Headquarters in Vienna from 21 to 25 August 2023 continuing to its seventh year. The annual gathering included directors and focal points from African and Asian national veterinary laboratories, international organizations (e.g., FAO, WOAH), FAO reference centers, and research institutions. Discussions covered IAEA and FAO activities in animal health, technological advancements in animal production and health, and VETLAB's role in capacity building, technology transfer, and knowledge sharing. The meeting promoted collaboration among international organizations, research centers, reference labs, and national diagnostic labs, addressing transboundary diseases, antimicrobial resistance, and quality control. Directors from VETLAB partner laboratories in various countries attended in person, with Ghana participating virtually. Representatives from AGAH, FAO, FAO regional office in Bangkok, WOAH, Pasteur Institute of Cambodia, and ERFAN (Italy) were also present. The meeting underscores the significance of supporting network members in developing reference materials, providing ring trials, and facilitating access to proficiency tests for various diseases to bolster the implementation of quality systems. Furthermore, it has encouraged developing and formalizing strategies to ensure the smooth acquisition of laboratory supplies and reagents for network members. The meeting also highlighted the importance of promoting active surveillance for relevant zoonoses in domestic, wild, and production animals, emphasizing the significance of health monitoring. Gathering around a shared table with colleagues from all over the globe is an experience that brings people closer together and is truly precious. We hope that the New Year brings you all the health, happiness, and success that you deserve, and we are grateful to everyone who helped make VETLAB a reality.

VETLAB Highlights

VETLAB harmonizing training with the FAO PPR Secretariat

In the second semester of 2023, VETLAB was involved in 1 national (Tanzania) and three regional training courses on PPR and respiratory diseases. This is a follow-up effort between the FAO-PPR secretariat and the APHL of the Joint FAO/IAEA Centre to harmonize the content and delivery of laboratory-related PPR training to member countries in line with PPR-GEP. All SOPs and reagents for training were supplied through VETLAB, and staff of the APHL attended the training in person or remotely. It was agreed that the manual used in this training would be consolidated and published as a handbook for PPR diagnosis.

Early and rapid diagnosis of PPR surveillance and post-vaccination surveillance of LSD in Indonesia

From 1 to 3 August 2023, the Diseases Investigation Centre in Subang-West, Java, collaborated with FAO (ECTAD Indonesia) to host a training on peste des petits ruminants (PPR) diagnosis. APHL provided remote expert lectures, engaging 26 veterinarians and 13 technicians on-site and remotely. Another 2-day online training, 19 to 20 September 2023, supported by APHL, focused on LSD diagnosis harmonization. It addressed key lab diagnostic issues and stressed the need for standardized SOPs in post-vaccination monitoring. VETLAB supplied reagents, materials, and SOPs for both training initiatives.

PPRV interlaboratory comparison (ILC)

The ILC for this year (2023) has concluded, and a report has been shared with the participants. In summary, there were 32 participants who tested the molecular panel from 29 different countries: 19 from Africa, 9 from Asia and 1 from Europe. Thirty-three participants tested the serological panel from 30 different countries: 20 from Africa, 9 from Asia and 1 from Europe. Of the 25 participants who provided results for both the serological and molecular panels, 16 (64%) scored 100%. The overall success rate was higher for the molecular panel than for the serological panel (75% vs 69.7%). Regarding the two molecular techniques applied in this ILC, the success rate using Real-time RT-PCR (91%) was higher than conventional RT-PCR (40%).

Development of a serological assay to differentiate between Capripoxvirus infected and vaccinated animals

Serological tests that differentiate infected from vaccinated animals (DIVA) are essential for effective disease surveillance. APHL has developed an iELISA capable of identifying antibodies against field infection, but not the vaccine, by targeting Capripoxviruses' B22R gene and gene products (lumpy skin disease, sheeppox, and goatpox viruses). The iELISA demonstrates >99% sensitivity and specificity for serum from animals infected with wild-type viruses without cross-reacting with anti-parapoxvirus antibodies. APHL plans for further comprehensive field validation of this assay, and another recently developed serological test for Capripoxvirus in collaboration with VETLAB partners.

VETLAB supported Libya in diagnosing the first LSD incursion in the country and characterizing local LSDV isolates

In 2023, suspicions of LSDV in Libya prompted an emergency response from VETLAB following an alert from the regional FAO office in Tunisia. VETLAB provided SOPs, reagents, and controls, enabling Libyan colleagues to diagnose LSDV promptly and report to WOAH. Post-diagnosis, Libya sought VETLAB's support to characterize local isolates. APHL analysis revealed that the Libyan LSDV belonged to the classical strain found in most African countries, distinct from recombinant and ancient LSDV strains recently re-emerging in South Asia.

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VETLAB
Network Bulletin

VETLAB Capacity Building Initiatives

Past Events

Training Course for Veterinary Diagnostic Laboratory Network Partners on Multiparametric Detection of Pathogens Causing Major Transboundary Animal Diseases and Zoonoses, 25 September to 6 October 2023, Seibersdorf, Austria.

This training aimed to enhance the capabilities of partners within the Veterinary Diagnostic Laboratory Network (VETLAB Network) in utilizing nuclear-derived/molecular and serological assays for differential diagnosis and syndromic surveillance of transboundary and zoonotic animal diseases. Thirty-one scientists from Asia and Africa participated, acquiring skills in detecting these pathogens, with a focus on multiplex molecular and serological assays led by experts from IRD France and the Joint FAO/IAEA Centres.

Various Training Courses on Detection and Differential diagnosis of PPR and Other Small Ruminants Respiratory Diseases

23 October to 3 November 2023, IRVT, Tunis, Tunisia (20 participants from 18 African countries)

4 to 8 September 2023, State Laboratory for Agriculture, Tbilisi, Georgia (14 participants from seven Central Asian/Asian countries)

5 to 9 November 2023, Directorate of Animal Wealth Laboratories (AWL), Amman, Jordan (13 participants from Jordan (10), Turkey (1) and Egypt (2)).

The events, organized in collaboration with the FAO PPR secretariat aimed to enhance the Veterinary Diagnostic Laboratory Network (VETLAB Network) and partner laboratories' capabilities for effective diagnostic testing across peste des petits ruminants (PPR) Global Eradication Programme phases. These training covered PPR, contagious caprine pneumonia (CCPP), and Capripox.

Training Course for Veterinary Diagnostic Laboratory Network Partners on Next Generation Sequencing Bioinformatics and Molecular Phylogeny, 11 November to 1 December 2023, Seibersdorf, Austria

Training focused on the genome analysis of animal pathogens, specifically targeting African swine fever (ASF), Capripox, peste des petits ruminants (PPR), and avian influenza viruses. Fifteen scientists from VETLAB partner laboratories in Asia and Africa, who were either already utilizing NGS or outsourcing NGS sequencing work, actively participated in this course.

VETLAB Highlights continued:

IAEA Supports Veterinary Laboratories in Controlling TADs, Allowing LANAVET (Cameroon) to Achieve Vaccine Production Records in 2023

The IAEA has supported laboratories for several decades in controlling transboundary animal diseases (TADs), including zoonoses. Beyond diagnosis and surveillance, the IAEA has played a crucial role in supporting vaccine production for preventive measures against animal diseases. Notably, the National Veterinary Laboratory (LANAVET) in Cameroon has benefitted significantly from IAEA support, achieving a remarkable milestone. Previously producing 15 to 20 million annual vaccine doses, LANAVET surpassed its record, making 35 million doses in 2023. These vaccines address diseases like anthrax, CBPP, PPR, lumpy skin disease, bovine pasteurellosis, black quarter, Newcastle disease, fowl cholera, and fowl typhoid. The Director General, Dr. Abel Wade, aspires to exceed 100 million annual vaccine doses with continued IAEA support in with the vision to of controlling TADs in Africa.

Follow up on the syndromic testing of abortifacient agent diseases in ruminants

VETLAB's partner laboratory in Botswana has developed a multiplex qPCR/HRM curve analysis for simultaneous detection of four zoonotic abortifacient agents in livestock, detailed in a published study (https://www.nature.com/articles/s41598-023-39447-1). Locally, the assay gained attention through presentations at the University of Botswana stakeholder engagements, including seminars, council meetings, and radio interviews. It was showcased at the UB Innovation Centre launch in May 2023 and discussed at the UB 40th Anniversary impact panel. The National Agricultural Research and Development Institute (NARDI) plans to launch the assay next year, initially for stakeholders and subsequently for public health institutions, promising valuable contributions to disease detection and control.

VETLAB Networking Activities

The Institut de la Recherche Veterinaire de Tunisie (IRVT) hosted the first post-COVID-19 VETLAB training event outside Austria

The Institut de la Recherche Veterinaire de Tunisie (IRVT) hosted a two-week training course in Tunis, Tunisia, from 23 October to 3 November 2023. The course, directed by Mrs. Aida Tlatli, aimed to enhance the capabilities of the Veterinary Diagnostic Laboratory Network (VETLAB Network) and partner laboratories in peste des petits ruminants (PPR) and small ruminants' respiratory disease diagnosis. The course was jointly supported by the Joint FAO/IAEA Center through VETLAB and FAO-PPR secretariat. The event was inaugurated by Prof. Hichem Ben Salem, President of IRESA, and attended by Dr. Sana Kacem, Tunisia's Chief Veterinary Officer. Participants from 18 African countries received hands-on training in PPR, contagious caprine pneumonia (CCPP), and Capripox, covering etiology, diagnosis, epidemiology, and molecular techniques. Positive feedback was received, and agreements were made for follow-up actions, including implementing techniques in participants' labs with VETLAB support. Collaboration with APHL of the Joint FAO/IAEA Center will focus on molecular epidemiological studies of animal poxviruses and peste des petits ruminants virus, with anticipated outcomes of significant contributions to national and regional disease control programs. IRVT showcased its capacity as a training center with administrative and technical support.

Highly pathogenicity avian influenza of different subtypes has been causing havoc in domestic poultry and wild birds globally. For up-to-date expert advice and information on avian influenza please visit the OFFLU network website at www.offlu.org

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Forthcoming Events

First Research Coordination Meeting on Innovative Nuclear and Related Molecular Approaches for Detection and Characterization of Antimicrobial Resistance in Animal Production Environment (D32043)

Kathiravan Periasamy

This will be the first meeting of the new coordinated research project (CRP) on innovative nuclear and related molecular approaches for detection and characterization of antimicrobial resistance in animal production environment and will take place at the IAEA Headquarters in Vienna, Austria, from the 15 to 19 April 2024. The purpose of the event is to discuss and finalize work plans of individual research contracts under the CRP that focusses on developing farm-level sampling and analytical methodologies for AMR detection and assess the efficacy of alternatives to antibiotic growth promoters (AGPs) as feed additives in animal production settings.

Second Research Coordination Meeting on Improving Efficiency of Animal Breeding Programmes Using Nuclear Related Genomic Information – Practical Applications in Developing Countries (D31030)

Victor Tsuma

The meeting is scheduled to take place at the IAEA Headquarters, Vienna, Austria, from 10 to 14 June 2024. The purpose of the event is to review the progress of individual research contracts, discuss the results, review and fine tune the workplans for planned activities, and determine timelines for publication and dissemination of output and results of the coordinated research project. agreement holders will review current status on animal breeding methodologies, and opportunities and challenges for application of nuclear and related genomic technologies for livestock improvement in developing country situations.

Second Research Coordination Meeting on Novel Test Approaches to Determine Efficacy and Potency of Irradiated and Other Vaccines (D32037)

Viskam Wijewardana and Carla Bravo de Rueda

The second research coordination meeting for the coordinated research project (CRP) D32037 on novel test approaches to determine efficacy and potency of irradiated and other vaccines will take place at the IAEA Headquarters in Vienna, Austria from 15 to 19 April 2024. The purpose of the event is to review the achievements of the coordinated research project and define the work plan for the next year of the project, with a special emphasis on animal experiments.

Past Events

APHL Assists Indonesia in Early Warning and Rapid Detection of PPR Disease

William Dundon

Between the 1 to 3 August 2023 the Diseases Investigation Centre, Subang-West, Java, Indonesia in collaboration with FAO (ECTAD Indonesia) held a training course on the molecular and serological diagnosis of Peste des Petits Ruminants (PPR). Expert lectures and Q&A sessions were provided remotely by two Animal Production and Health Laboratory (APHL) experts, in addition to reagents and material (molecular and serological test panels).

Participants purifying RNA samples for PPRV diagnosis

Twenty-six veterinarians and thirteen laboratory technicians participated both remotely and on-site preforming molecular and serological tests. VETLAB Network and PPR PUI projects contributed by US and Japan Governments, respectively, supported this event.

Webinars in Russian, Spanish, and English on Avian Influenza (AI)

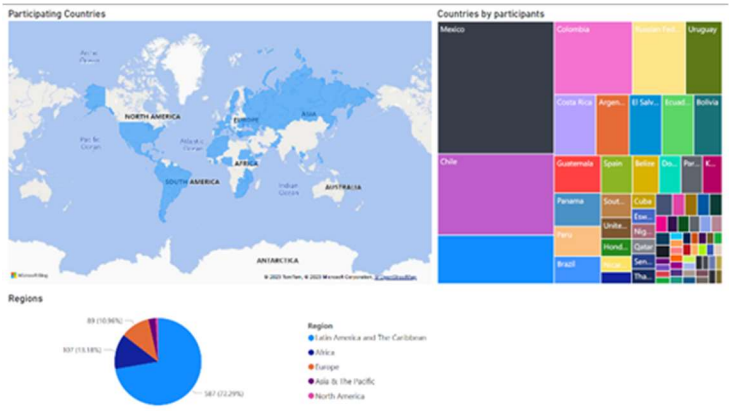
Carla Bravo de Rueda

Webinars (in Spanish, English, and Russian) organized jointly by the Joint FAO/IAEA Centre and FAO Animal Production and Health division (NSA) took place in the first half of 2023. The webinars were designed to focus on avian influenza (AI) surveillance, laboratory diagnostics, and biosafety. The purpose of these webinars was to clarify technical aspects, as well as inform laboratories about the sampling and laboratory procedures to be applied in the field and in the laboratory.

The webinar (in Spanish) focusing on vaccination strategies (432 participants) against AI was aimed at helping Latin

America and the Caribbean veterinary laboratories to define vaccination strategies including the selection of AI vaccines, under careful examination of experts working closely with other international organizations.

The webinars were successfully delivered with the contributions of approximately 25 AI international and regional experts and was attended by more than 855 participants from Latin America and Caribbean, Europe, Asia, Central Asia and Caucasus, and Africa.



Summary of global participation of the AI webinars

Click here to read more(in Spanish)

Regional Virtual Training Course on Avian Influenza in Africa (INT5157)

Carla Bravo de Rueda

On 21 June 2023, the IAEA, through its Division for Africa and the Section for Animal Production and Health, organized a webinar entitled 'Avian Influenza in Africa – Lessons Learnt on Preparedness and Control of Avian Influenza' for the ZNLs to help member states to control this disease of public health concern. This 3 hour virtual webinar addressed question about Highly Pathogenic Avian Influenza (HPAI) diagnosis in Africa with the aim to provide technical assistance to all ZNLs and help solve doubts about the incursion of HPAI in the region and its control methods. Experts from the Istituto Zooprofilattico Sperimentale delle Venezie (Italy), FAO-Animal Production and Health Division (Italy), Central Laboratory for Animal Diseases (Côte d'Ivoire), Laboratory for Veterinary Quality Control on Poultry Production (Egypt), National Veterinary Research Institute (Nigeria) and the Global Health Programme (UK) shared their teachings and approaches to tackle HPAI before its incursion in the region.

The target audience for the webinars were animal health laboratory staff and ZODIAC National Laboratories from Africa and Asia, there were around 100 participants. The event was held in English.

National Training Course on Artificial Insemination for Livestock Breeding in Sierra Leone (SIL5022)

Victor Tsuma

The national training course was held at Njala University, Sierra Leone from 17 to 28 July 2023. In attendance were 21 participants consisting of various animal industry stakeholders from various parts of Sierra Leone. Dr. James Ombura, a livestock development expert from Kenya, was the expert trainer, providing guidance, materials, and skills to all trainees.

During the 10-day training course, lectures, practicals, demonstrations, and hands-on artificial insemination (AI) practice in cattle were conducted. The course content included an overview of bovine anatomy and reproductive physiology as it relates to AI; manipulation of the oestrous cycle to optimize breeding; semen collection, processing, and preservation; AI as a breeding tool; AI equipment, semen, and liquid nitrogen handling; bovine AI step-by-step; standard operating procedures for successful AI practice; and trouble-shooting causes of AI failure.

Participants at the national training course on artificial insemination in Sierra Leone with the project counterpart Prof. Suluku (front row third from left) and the facilitator Dr. Ombura (front row second from right)

Midterm Coordination Meeting of the Regional Project on Integrated Soil Cropping Livestock Production Systems (RAF5090)

Victor Tsuma

In many African countries, food and nutrition security face the challenges of producing enough food to meet increasing demand in the wake of a growing human population, climate change, natural resource depletion, and biodiversity loss. Sustainable intensification of agriculture would optimise resource-use efficiency and increase farm productivity. The Joint FAO/IAEA Centre is supporting a regional project to evaluate, develop, and implement sustainable farming models using an integrated soil, crop, and livestock production system approach, where nuclear science and technology would be applied for increasing agricultural productivity while addressing climate change and its impact at the farm level. A project midterm

coordination meeting was held from 30 October to 3 November 2023 in Cairo, Egypt to:

I. discuss views, experiences, and impact on farm productivity of implementation of integrated soil-crop-livestock production system (ISCLPS) in different African countries,
II. discuss the major components of ISCLPS and how they beneficially interact to address food security and environmental challenges,
III. share and discuss technologies and strategies needed for successful implementation of ISCLPS, and
IV. review organizational and/or institutional support and way forward for the successful implementation of the ISCLPS.

Twenty-four soil, crop, and livestock experts drawn from government ministries, research institutions, national agricultural development boards, and universities from 13 African countries (Algeria, Egypt, Eswatini, Ethiopia, Ghana, Kenya, Libya, Mauritania, Mauritius, Morocco, Nigeria, Rwanda, South Africa) attended.

Participants at the mid-term coordination meeting of the regional project on Integrated Soil-Cropping-Livestock production systems, Cairo, Egypt

Second Coordination Meeting of the Regional ARCAL Technical Cooperation Project on Rainbow Trout Farming (RLA5086)

Kathiravan Periasamy and Carla Bravo de Rueda

The second coordination meeting of the Regional ARCAL Technical Cooperation Project RLA5086: Decreasing the mortality rate of rainbow trout associated with infectious pancreatic necrosis virus and emerging diseases using molecular and OMIC techniques was held virtually from 6 to 8 November 2023. National coordinators from eight participating countries (Argentina, Brazil, Chile, Ecuador, Mexico, Panama, Peru and Uruguay), three observers, and three IAEA staff members attended the meeting.

Aquaculture is one of the fastest growing sectors and is expected to grow further to secure food for the growing human population. The culture of rainbow trout (Oncorhynchus mykiss) is one of the main aquaculture activities in the Latin America and the Caribbean (LAC) region. Aquaculture is developed mostly by rural communities with low economic resources. Consequently, in the area of aquaculture, many countries in the region lack adequate infrastructure, qualified personnel, epidemiological surveillance, and adequate sanitary controls.

Second virtual coordination meeting of the regional ARCAL RLA5086 Technical Cooperation project

The region is experiencing increasing mortality rates in trout farming due to increased production stress on the animals and subsequent susceptibility to infectious agents. In addition, the incursion of new pathogens in the absence of timely diagnosis is affecting aquaculture production. There is also a considerable risk of introducing vertically transmitted foreign diseases, due to the fact that most countries base their production on commercial embryonated eggs imported from elsewhere. The project aims to strengthen trout farming in the region by improving the diversity of genetic base for better production while reducing high mortality rates caused by the infectious pancreatic necrosis virus (IPNV) and other emerging diseases. Three major areas are targeted for building national/regional capacities: (i) assessing the biodiversity of rainbow trout populations in Latin America, (ii) strengthening capacities in the use of RT-PCR (reverse transcription-polymerase chain reaction) and genome sequencing approaches for early and rapid diagnosis of aquaculture pathogens, and (iii) application of genomic technologies for breeding trout with enhanced host resistance against infectious diseases.

During the meeting, the national coordinators presented their country reports. They updated attendees on the status of trout farming, genetic base (strain/varieties) of trout used for farming, status of IPNV infection, diagnostic methodology used, other infections detected/reported (bacteria, fungi, and parasites), and breeding for genetic disease resistance. With the assistance of IAEA technical officers, they deliberated and finetuned the technical work plan for the next two years of the project that includes: (i) sampling for disease surveillance and methods of pathogen detection, (ii) collection of data on disease incidence, (iii) sampling trout for biodiversity assessment, and (iv) genome wide association study on resistance to IPNV. During the meeting, a strategic outline for surveillance of disease-causing pathogens in aquaculture was formulated. The participants also finalized the technical areas to be targeted for regional training courses under the project and the schedule for their implementation.

Third Virtual Coordination Meeting on the use of Stable Isotopes to Trace Bird Migrations and Molecular Nuclear Techniques to Investigate the Epidemiology and Ecology of the Highly Pathogenic Avian Influenza Virus (D32034)

Ivancho Naletoski and Charles Lamien

The third coordination meeting for the coordinated research project (CRP) D32034 took place between 4 to 8 December 2023. The meeting reviewed the progress regarding the use of stable isotopes to trace bird migrations and molecular nuclear techniques to investigate the epidemiology and ecology of the Highly Pathogenic Avian Influenza Virus. The purpose was to review the samples collected for analysing avian influenza and other diseases transmitted by migratory birds, to discuss the results obtained from the stable isotope analysis, and to discuss the overall project achievements and considerations for the future.

National Training Course on Bioinformatics Analysis for Genomic Characterization of Livestock Biodiversity (SEN5042)

Kathiravan Periasamy

Under the IAEA technical cooperation project SEN5042: Using nuclear and related techniques in improving the productivity of domestic ruminants, a national training course on "Bioinformatics analysis for Genomic Characterization of Livestock Biodiversity" was organized from 13 to 24 November 2023 at Laboratoire National de L'Elevage et de Recherches Veterinaires (LNERV), Dakar, Senegal. Twelve participants working at various research institutions of Institut Senegalais de Recherches Agricoles (ISRA) in Senegal attended the training course.

National training course on bioinformatics analysis of genomic data in Senegal

The course included lectures and practical hands-on training covering: (i) FAO Guidelines on Genomic Characterization of Livestock, (ii) DNA marker tools for assessing livestock biodiversity, (iii) principles of genome wide typing and extraction of genome wide SNP genotype data, (iv) executing basic Unix commands and R scripts, (v) introduction to managing large sets of data using command line platforms: (vi) introduction to PLINK and Data Quality Control (pruning genome wide single nucleotide polymorphic data), (vii) estimation of basic measures of genomic diversity, (viii) estimation of inbreeding and effective population size using genomic data, and (ix) assessment of population structure and estimation of genetic admixture.

Dr. Stephane Arnaud Tepsoba (Institut de l'Environnement et des Recherches Agricoles (INERA), Ouagadougou, Burkina Faso) served as an expert lecturer for the training course. The training is expected to help improve the national capacity on applying genomic technologies for developing baseline information and characterization of indigenous livestock breeds of Senegal. The training will also help strengthen human resource capacity for effective implementation of the National Action Plan on Animal Genetic Resources in Senegal.

Technical Workshop on Cryopreservation of Bovine Semen for Artificial Insemination and Conservation of Indigenous Zebu and Taurine Cattle (SEN5042)

Kathiravan Periasamy

Under the IAEA technical cooperation project SEN5042: Using nuclear and related techniques in improving the productivity of domestic ruminants, a technical workshop on "Cryopreservation of bovine semen for artificial insemination and conservation of indigenous zebu and taurine cattle" was organized from 15 to 16 November 2023 at Centre National d'Amelioration Genetique (CNAG), Dahra, Senegal.

Participants working on collection, evaluation, packaging, and cryopreservation of bull semen during the technical workshop

Eight participants, including staff and students working at the frozen semen center of CNAG, attended the training. The workshop provided practical hands-on training to the participants on: (i) collection and evaluation of semen from indigenous zebu and taurine cattle, (ii) preparation of extenders and dilution of semen, (iii) packaging of semen, (iv) pre-freeze evaluation of sperm motility, (v)

cryopreservation of semen, and (vi) post-thaw quality evaluation of spermatozoa. Staff from the Animal Production and Health Laboratory (APHL) served as an expert resource for the workshop. The workshop is expected to help improve the capacity of CNAG to successfully cryopreserve bovine semen for enhancing artificial insemination services and breeding of cattle in Senegal. The workshop will also help the ongoing national programme on cryo-conservation of indigenous zebu and taurine cattle in the country.

National Training Course on Validation of ELISA Method and Calculation of Uncertainties (ALG5032)

Ivancho Naletoski

A national training course on validation of ELISA method and calculation of uncertainties was delivered from 18 to 21 September 2023 at the Central Veterinary Laboratory of Alger (LCV) in Algeria. Two expert lecturers from the reference laboratory ANSES (National Agency for Food Safety, Environment and Work) contributed by sharing experiences and best practices. All staff members of LCV, responsible for the implementation and maintenance of the ISO 17025 standard for testing and calibration, were invited to attend the course.

Workshops on Laboratory Methods for Peste des Petits Ruminants (PPR) Diagnosis

William Dundon

From 4 to 8 September 2023, a laboratory expert from the Animal Production and Health Laboratory (APHL) gave a training on "Methods for peste des petits ruminants (PPR) diagnosis" at the State Laboratory for Agriculture, Tbilisi, Georgia. The workshop was co-organised by APHL together with the FAO Regional Office for Europe and Central Asia (REU) and the FAO PPR Global Eradication Programme (GEP) Secretariat. Laboratory diagnosticians from Armenia (2), Azerbaijan (2), Georgia (4), Kyrgyzstan (1), Tajikistan (2), Uzbekistan (2), and Mongolia (2) attended the workshop.

Participants of the PPR diagnosis training - Tbilisi (left), Georgia (right)

A second PPR laboratory diagnosis workshop was conducted at the Directorate of Animal Wealth Laboratories (AWL), Amman, Jordan from 5 to 9 November 2023. It was jointly organized by APHL, Joint FAO/IAEA Centre, the FAO PPR Secretariat and the IAEA coordinated Veterinary Diagnostic Laboratory (VETLAB) Network framework of the PPR Global Control and Eradication Strategy. Experts from APHL and FAO provided hands on training on molecular and serological diagnostic methods for PPR diagnosis. The sequencing protocols for characterization of PPRV and the workflow from amplicon preparation, sequencing data analysis to phylogenetic analysis was demonstrated. to Thirteen participants from Jordan (10), Turkey (1) and Egypt (2) attended the workshop.

Regional Training Course on Next Generation Sequencing (NGS) using Illumina Platform (RAS5085)

Ivancho Naletoski and Charles Lamien

The training course was delivered between 10 to 14 September 2023 in Dhaka, Bangladesh, at the Bangladesh Livestock Research Institute (BLRI). Counterparts from the laboratories of the Asian region received theoretical and practical lectures on the principles and application of the New Generation Sequencing (NGS) technologies, using the Illumina platform.

The course covered: i) sample preparation and inactivation [a) host nucleic acids (NA) removal, b) extraction of NAs, c) biological safety of extracted NAs for international, d) use / benefit of specific procedures (RNA based capture, SISPA etc.), and e) PCR amplification-based procedures for specific pathogen (primers used for FMD or LSDV)], ii) library preparation, iii) Illumina platform setup and operation, and iv) suggestions on bioinformatic workflows (Linux based approach and Galaxy platform approach) for Illumina platform based NGS.

Participants of the Regional Training Course on Next Generation Sequencing (NGS) using Illumina Platform in Dhaka, Bangladesh

The knowledge and skills obtained at the training course are expected to significantly facilitate participation of the laboratories in the serviced based NGS (optimized under the coordinated research project D32036 and will be free of charge for the end user laboratories).

The training was supported by four international experts from advanced veterinary laboratories. Participants included 38 individuals.

Second Research Coordination Meeting on the Application of Advanced Molecular Characterization Technologies Through the Veterinary Diagnostic Laboratory (VETLAB) Network (D32036)

Ivancho Naletoski and Charles Lamien

The second research coordination meeting for the coordinated research project (CRP) D32036 occurred from 21 to 25 August 2023, at the IAEA Headquarters in Vienna, Austria. Fifteen partners of the CRP (agreement holders (4), technical partners (3), and research partners (8)) attended the meeting. The discussions focused on the progress of the development of the standard operating procedures (SOPs) for sample preparation, submission, and data processing (bioinformatics) for the service based Whole Genome Sequencing (WGS). The meeting was held simultaneously with the annual meeting of the Directors of the VETLAB Network laboratories in order to review their priorities.

The partners of the CRP D32036 together with the Directors of the VETLAB Network laboratories

Regional Training Course on Capturing and Sampling Wildlife Animals (RER5027)

Ivancho Naletoski

A regional training course on capturing and sampling wild animals was organized at the Veterinary Specialized Institute Kraljevo (VSI KV), Kralijevo, Serbia, from 9 to 13 October 2023. The course covered: i) theoretical presentation of international regulations and recommendations for capturing of wild animals, ii) practical presentations from experts about strategies of capturing different wildlife animal species (bats, wild ruminants, wild carnivores, rodents, wild pigs), iii) practical presentation of placing appropriate traps for different wildlife animal species, iv) practical presentation of handling (including sedation) of captured wildlife, and v) practical presentation of collecting appropriate samples from captured wild animals. Domestic and international experts supported the implementation of the training course with 22 countries and 39 participants from 39 different institutions joining the training course.

Participants at the RER5027 regional training course on capturing and sampling wildlife, with support of an international expert during the installation of bat traps in the forest.

Regional Training Course on the Use of iVetNet Information Platform in the Implementation and Maintenance of the ISO 17025 Standard (RAS5085)

Ivancho Naletoski

iVetNet is an online information platform developed by the Animal Production and Health Section (APH) under the past coordinated research project (CRP) D32032. It contains multiple modules for collection and storage of records relevant for ISO 17025, such as the personal records, infrastructure, and equipment records and module for exchange of validated standard operating procedures with QA/QC records. These are reviewed through a system of queries and reports to help the quality management of the local laboratories to monitor and coordinate the quality management system.

Records of the local laboratories are linked to the module for planning and implementation of the APH subprogramme. Additionally, it links institutions and laboratory staff to specific activities of the subprogramme (such as events, procurement, publications, etc.). Such a link enables interactive communication (exchange of information) between the APH subprogramme and the counterpart laboratories, as well as between individual laboratories.

The training course on iVetNet was held between 12 to 16 November 2023 in Doha, Qatar. The participants at the training course received detailed, step-by-step information on the use of iVetNet at the local laboratories.

The training course was supported by one experienced expert and the technical officer of the project. Thirty-two participants belonging to 21 institutions from 18 countries participated in the training course.

Regional Training Course on Serological Diagnostics (RAF5089)

Carla Bravo de Rueda

A regional training course at the Office National de Recherches et de développement de l'élevage (ONARDEP), Nouakchott, Mauritania, took place from 10 to 14 July 2023. The purpose of this event was to train participants in serological techniques for zoonotic and animal diseases, as well as enhancing national and regional capacities in the surveillance, detection, and control of emerging or re-emerging animal and zoonotic diseases.

In total, 22 participants from eight countries, including four locals, participated in this event. They were trained in the use of ELISA for the diagnosis of foot-and-mouth-disease (FMD) and Rift Valley fever (RVF). This training had a theoretical and laboratory component. Trainers from the Pasteur Institute Dakar, Senegal, demonstrated the use of the ELISA technique in diagnostics and the importance of accurate results and consequent reporting. Official Mauritanian authorities opened the event which was showcased on national TV.

Training involved theoretical and practical sessions in the ELISA technique using FMDV and RFV as priority diseases

Regional Training Course on Molecular Characterization of Brucella (RLA5085)

Carla Bravo de Rueda

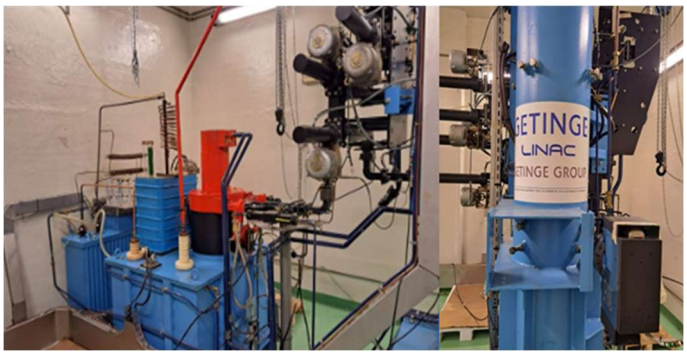
A regional training course on culture and characterization of the genus *Brucella* species circulating in Latin America and the Caribbean (LAC) took place at the Servicio Nacional de Salud Animal (SENASA), Ministerio de Agricultura, San Jose, Costa Rica, from 27 November to 1 December 2023.

Eighteen LAC countries participated in this event, including the Costa Rican hosts. Trainers from the host laboratory and from the Centro de Investigación y Tecnología Agroalimentaria (CITA), Zaragoza, Spain, shared their knowledge using theoretical modules on the diagnostics of Brucellosis including isolation, MALDI-TOF, Bruce-ladder, MVLA-16, and NGS. LAC participants brought national samples to do a regional investigation on the current circulation of *Brucella sp*. Participants used CITA and Farrell selective media for bacterial culture and molecular tools such as PCR Bruce-ladder, MVLA-16, and sequencing for the characterization of bacteria. A field trip to a number of dairy farms was also organized by the hosts to better understand the control of this disease at the national level.

Expert Visit Establishing a National Certified Pipeline to Produce Aquaculture Vaccines by Irradiation (TUN5032)

Richard Kangethe and Viskam Wijewardana

An expert visit to a Tunisian counterpart involved in the TC Project TUN5032 that aims to establish irradiated vaccines for fish and other aquatic organisms was carried out focusing on Nodavirus infections in Seabream and Seabass, two species that account for over 60% of the entire aquaculture output in the Mediterranean. Working visits were made to three participating national institutes, one engineering school, a collaborating biotech firm and a commercial fish producer on site in the sea that produces approximately 8000 tonnes of fish annually. At the National Institute of Marine Sciences and Technologies (INSTM) in Salambo, Nodavirus from routine sample testing is propagated *in vitro* using E-11 cells in Leibovitz's L-15 medium at 25°C. Cultured virus is then titrated and prepared for irradiation at different doses using the high energy electron beam facility at the National Centre of Nuclear Sciences and Technologies (CNSTN). Irradiated virus is subsequently titrated to calculate the D10 (dose required to reduce viral titre by one log) and inactivation dose for immunisation. These experiments are currently ongoing at both institutes with ideas on how to improve reproducible doses for calculating D10 discussed. It was also suggested that the Animal Production and Health Laboratory (APHL) begin to explore *in vitro* methods that can be adopted to measure and compare fish immune responses to irradiated versus chemically treated viral samples and for the preservation of viral antigen immunogenicity after irradiation.



High energy electron beam generator located in CNSTN

At the institute Pasture in Tunis (IPT), virus-like particles (VLPs) expressed in bacteria are being developed as an alternative to using live virus for vaccine experiments. An innovation that uses radiation-inducible promoters in *E. coli* and *Deinococcus grandi* were delivered from Seibersdorf and tested using UV at different exposures. Initial results

looked promising but require a range of confirmation experiments. Materials for the purification and refolding of expressed VLP were also delivered to test initial samples identified. At the Engineering School of Communication of Tunis (Sup'Com), a faculty competition on describing how AI can be used to predict the immunogenicity and reactogenicity of irradiated vaccines was held with the top three ideas awarded. A talk about APHL activities on immunology and practical aspects to consider when using Irradiation as a tool for developing Livestock Vaccines was also presented.

Regional Training Course on Validation of Diagnostic Techniques According to ISO 17025 (RLA5085)

Carla Bravo de Rueda

A regional training course on diagnostics test validation using analytical and quantitative parameters under the ISO 17025 standards took place at the IAEA Headquarters in Vienna, Austria from 14 to 18 August 2023.

Nineteen participants from 18 Latin-American and Caribbean (LAC) countries participated in this event. They were trained on the use of statistical methods to calculate diagnostic parameters such as: sensitivity (Se), specificity (Sp), cut-off, repeatability, reproducibility, serial/parallel testing, predictive values, likelihood ratios, apparent and true prevalence, and receiver operating characteristic curve (ROC).

Hands-on calculation modules using real diagnostics data from LAC laboratories

MedCalc and Bayesian latent class models (BLCM) were introduced to the participants who used real databases for the calculation of validation parameters. Additionally, the participants acquired knowledge on the required steps to accredit their diagnostic tools according to the World Organization for Animal Health (WOAH) standards. Trainers from the WOAH Reference Laboratory for Diagnostic Test Validation Science at the Australian Centre for Disease Preparedness (former CSIRO) gave both theoretical and hands-on instructions.

Veterinary Diagnostic Laboratory (VETLAB) Network Coordination Meeting with Directors of African and Asian Veterinary Laboratories

Charles Lamien

The 7th coordination meeting of the VETLAB Network took place from 21 to 25 August 2023 at the IAEA Headquarters, Vienna, Austria.

The annual gathering included directors and focal points from African and Asian national veterinary laboratories, international organizations (e.g., FAO, WOAH), FAO reference centers, and research institutions. Discussions covered IAEA and FAO activities in animal health, technological advancements in animal production and health, and VETLAB's role in capacity building, technology transfer, and knowledge sharing. The meeting promoted collaboration among international organizations, research centers, reference labs, and national diagnostic labs, addressing transboundary diseases, antimicrobial resistance, and quality control. Directors from VETLAB partner labs in various countries attended in person, with Ghana participating virtually. Representatives from AGAH, FAO, the FAO regional office in Bangkok, WOAH, Pasteur Institute of Cambodia, and ERFAN (Italy) were also present.

The meeting underscores the significance of supporting network members in developing reference materials, providing ring trials, and facilitating access to proficiency tests for various diseases to bolster the implementation of quality systems. Furthermore, it has encouraged developing and formalizing strategies to ensure the smooth acquisition of laboratory supplies and reagents for network members.

The meeting also highlighted the importance of promoting active surveillance for relevant zoonoses in domestic, wild, and production animals, emphasizing the significance of health monitoring.

Regional Training Course on Diagnostic Techniques for African Swine Fever Virus (RLA5085)

Carla Bravo de Rueda

A regional training course on the use of quantitative PCR and ELISA for the diagnostic of African Swine Fever (ASF), took place at the Laboratório Federal de Defesa Agropecuária (LFDA-MG) in Minas Gerais, Brazil, from 16 to 20 October 2023.

Thirty participants, including 11 nationals, participated in this event. Swine disease professionals from: Honduras, Venezuela, El Salvador, Uruguay, Chile, Brazil, Costa Rica, Nicaragua, Ecuador, Peru, Mexico, Colombia, Cuba, Panama, and Paraguay participated in the event lead by four

Spanish trainers from the National Institute of Agricultural and Food Research and Technology (INIA), Center for Research in Animal Health (CISA). The participants were divided into four groups for their participation in theoretical and laboratory modules that used INIA/CISA blinded ASFV samples panels for their analysis using qPCR and ELISA. The FAO Regional Office for Latin America and the Caribbean gave an overview of the situation of ASF in Latin America and the Caribbean which opened a discussion for future steps in the control of ASF in the region.

Participants at the INIA/CSIA ASF diagnostics training

Nuclear Technology Application Industry Development Forum of China

Bo Liu

An APHL expert on animal vaccine regulatory affairs presented “The Application of Nuclear Technology in Animal Vaccine Development” on 16 November 2023 at the Nuclear Technology Application Industry Development Forum of China Nuclear Energy High-quality Development Conference/Shenzhen International Nuclear Energy Industry Innovation EXPO. The forum brought together experts from the Chinese government, relevant research institutes, and industry to discuss the wide range of applications and future potential of nuclear technology.

The Joint WHO Joint External Evaluation (JEE) Mission in Indonesia

Luca Porfiri

The Joint WHO Joint External Evaluation (JEE) mission in Indonesia, led by a subgroup of the JEE team, visited facilities in Yogyakarta, Indonesia, focusing on biosafety, biosecurity, zoonotic diseases, and surveillance. An expert from the Animal Production and Health Laboratory participated to provide expertise in the One Health area. The first facility visited was the Disease Investigation Centre (DIC) Wates, responsible for monitoring, surveillance, and outbreak investigation activities in three provinces. The centre has invested in quality management and has obtained five ISO accreditations. The DIC Wates has also participated in a back-to-back FAO Laboratory Mapping Tool (LMT) programme and is part of a WOAH-Twinning laboratory programme with Geelong, the Australian Animal Health Laboratory (AAHL).

The second visit was to the "Alert Village" in Kulon Progo District, where the JEE expert team met with field epidemiologists who explained the village's status as a zoonotic hotspot. The village has reported cases of MERS, rabies, leptospirosis, anthrax, and H1N1, leading to a successful One Health approach to mitigate anthrax cases. The JEE team visited the BRIN Institute in Cibinong, which includes the Research Centre for Veterinary Science, the Research Organization for Health, and the National Research and Innovation Agency. The institute has seven research groups, including Zoonosis, Emerging/Re-emerging and Neglected Diseases, Vaccine and Animal Medicine, Livestock Health and Veterinary Public Health, Disease Detection and Vector and Animal Health Control, Veterinary Toxicology and Pathobiological Science, Aquatic Animal Health, and Veterinary Services.

The JEE expert from APHL explaining the JEE process and relevant technical areas to local stake holders

Training Course for Veterinary Diagnostic Laboratory (VETLAB) Network Partners on Multiparametric Detection of Pathogens Causing Major Transboundary Animal Diseases and Zoonoses

Charles Lamien

This training course was held from 25 September to 6 October 2023 at the IAEA Laboratories in Seibersdorf, Austria. The training aimed to enhance partners' capabilities within the VETLAB Network in utilizing nuclear-derived/molecular and serological assays for the differential diagnosis and syndromic surveillance of significant transboundary and zoonotic animal diseases.

Over two weeks, participants acquired skills in detecting and differentially diagnosing transboundary and zoonotic animal pathogens, employing molecular and serological approaches.

The first week focused on probes-based and HRM-based approaches for syndromic testing and multiparametric detection. Practical examples were provided on respiratory pathogens of small ruminants, haemorrhagic diseases of swine, avian diseases, pox diseases of ruminants and camels, and abortive diseases of ruminants.

The second week was dedicated to multiplex serological assays utilizing the Luminex platform, featuring examples of ebolaviruses, arboviruses, and monkeypox viruses. Additionally, nanopore-based methods for zoonotic respiratory viruses were covered.

Thirty-one scientists from VETLAB partner laboratories in Asia and Africa actively participated in this course. The lectures were delivered by experts from Institute for Research and Development (IRD) France and the Joint FAO/IAEA Centre.

Training Course on the Detection and Differential Diagnosis of PPR and Other Small Ruminants Respiratory Diseases

Charles Lamien

The training course was organized by Institute for Veterinary Research (IRVT) Tunisia from 23 October to 3 November and held at their laboratories in Tunis, Tunisia.

The event's objective was to enhance the capabilities of the Veterinary Diagnostic Laboratory (VETLAB) Network and partner laboratories engaged in the Peaceful Uses Initiative peste des petits ruminants (PPR) project. This enhancement will empower them to conduct diagnostic tests effectively across various PPR Global Eradication Programme phases.

For two weeks, participants underwent training on diagnosing and monitoring peste des petits ruminants (PPR) and other respiratory diseases in small ruminants. The training included hands-on experience in diagnostics, bioinformatics, and molecular epidemiology of PPRV, MCCP, and Capripoxvirus. The first week of training was focused on peste des petits ruminants, while the second covered contagious caprine pneumonia (CCPP) and Capripox. The curriculum included the etiology, clinical and laboratory diagnosis, epidemiology, and molecular epidemiology, encompassing phylogenetic reconstructions.

Twenty participants representing 18 African countries attended the course. Experts from CIRAD, IRVT Tunisia, and the Joint FAO/IAEA Center delivered the training.

Training Course for Veterinary Diagnostic Laboratory (VETLAB) Network Partners on Next Generation Sequencing Bioinformatics and Molecular Phylogeny

Charles Lamien

During the two-week programme, from 20 November to 1 December 2023 held at the IAEA Laboratories, Seibersdorf, Austria, participants were trained on sample and library preparation, sequencing using Next Generation Sequencing (NGS) and Nanopore technology, and sequencing data analysis. The training focused on the genome analysis of animal pathogens, specifically targeting African swine fever (ASF), Capripox, peste des petits ruminants (PPR), and avian influenza viruses.

The training programme was divided into two weeks, with the first week dedicated to wet laboratory work involving the Ion S5 NGS platform and the Nanopore MinION device. The second week focused on NGS and Nanopore data analysis and comparative sequence analysis, including phylogenetic reconstructions.

Additionally, the training introduced participants to viral family-based pathogen screening and discovery strategies developed at the Animal Production and Health Laboratories. These strategies involve a combination of multiplex PCR targeting viral families and nanopore sequencing.

Fifteen scientists from VETLAB partner laboratories in Asia and Africa, who were either already utilizing NGS or outsourcing NGS sequencing work, actively participated in this course. The lecturers for the course were from the Joint FAO/IAEA Centre.

IAEA- CEA (The French Alternative Energies and Atomic Energy Commission) Seminar on ZODIAC Cooperation Programme

At the IAEA headquarters, Vienna, Austria, a scientific meeting was organized between the Animal Production and Health section and the French Alternative Energies and Atomic Energy Commission (CEA). The CEA is a key player in research, development, and innovation in four main areas: nuclear and renewable energies, technological research for industry, defence and security, and fundamental research in the physical sciences and life sciences.

Three main areas of possible collaboration were defined with the partners from CEA, as follows: i) overarching collaborative models to be pursued, ii) detection and characterization technologies for animal and zoonotic pathogens, and iii) vaccine development and immunology.

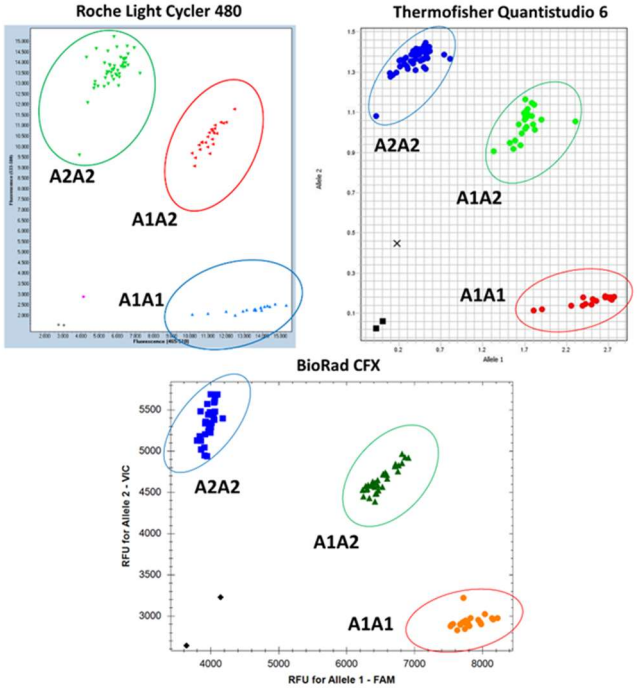
CEA-IAEA Seminar on ZODIAC Cooperation Programme

Research Activities of the Animal Production and Health Laboratory

Animal Genetics

Development and Validation of a Cost-effective DNA Test to Identify Cows Producing A1/A2 Milk (D31030)

Considering the human health significance of beta casein milk protein variants (A1 milk vs A2 milk), there has been considerable interest in several FAO/IAEA member states to identify and breed cows that produce A2 milk. During 2023, the Animal Production and Health Laboratory (APHL) initiated the development of a simple, cost-effective DNA test that can help farmers and breeders to differentiate cows that produce A1 and A2 milk. Earlier in the year, a competitive allele specific polymerase chain reaction (PCR) assay based on fluorescence resonance energy transfer (FRET) chemistry was designed to genotype A1/A2 polymorphism.

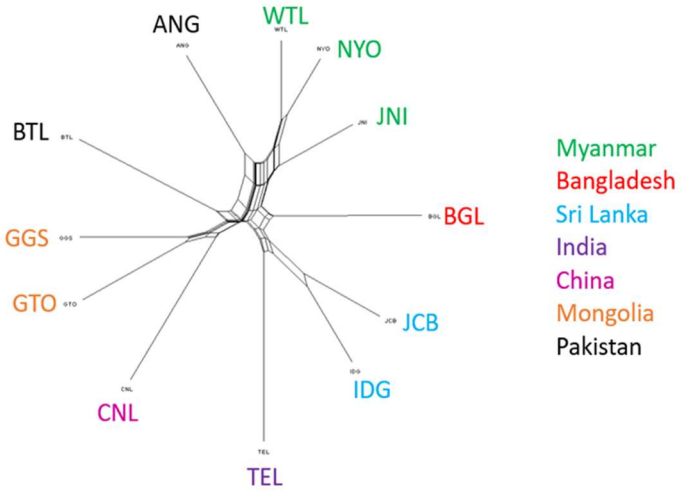


Comparative evaluation of A1A2 genotyping across three real time PCR platforms

The assay development was successful, and its efficacy was validated across three real time PCR platforms: viz. BioRad CFX, Roche Light Cycler, and ThermoFisher Quantstudio6. A total of 352 cattle samples belonging to seven breeds were genotyped across the above-mentioned platforms. All the samples were subjected to targeted sequencing of partial CSN2 gene for cross-verification and validation. The results showed successful and precise genotyping of 352 (100%), 351 (99.7%), and 349 (99.1%) samples in BioRad CFX, Roche Light Cycler, and ThermoFisher Quantstudio6 platform, respectively. The validation process of the assay is complete and is ready for transfer to member states in need.

Genomic Diversity of Indigenous Myanmar Goats and their Relationship with South Asian Goat Populations

The goat population in Myanmar is around 10.3 million. While these goats are primarily reared for meat consumption, some are reared for milk production. Myanmar is characterised by hills and valleys, and the goats are widely spread all over the country surviving in a dry and mountainous environment. The four most common regions where goats are raised in Myanmar are: Magway, Mandalay, Sagaing, and Yangon. Myanmar's goat population is characterised by three major breeds: Jade Ni (JNI), Nyaung Oo (NYO), and Waithar Li (WTL). These breeds have uniform flocks distributed across the above-mentioned regions. The Animal Production and Health Laboratory (APHL) supported the study on genomic characterization of indigenous Myanmar goats and assessed their relationship with other South Asian goat populations. A total of 569 goats were genotyped using the goat array (Axiom Goat Genotyping v1 array) that consisted of 58655 single nucleotide polymorphic (SNP) markers. The observed heterozygosity ranged from 0.360 for JNI, 0.361 for WTL, and 0.362 for NYO goat breeds — with an average of 0.372 across South Asian breeds. Genetic distance estimations showed the uniqueness of Myanmar goats and shared ancestry with Angora goat breed. The within and between breed genetic diversity metrics estimated in the study is expected to help with formulating effective strategies for the management of goat genetic resources in Myanmar.



NeighborNet tree showing relationship between Myanmar and other South Asian goat breeds

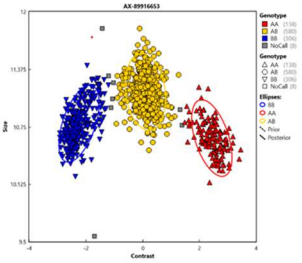
Genome Wide Association Study on Resistance to Infectious Pancreatic Necrosis Virus (IPNV) in Chilean Rainbow Trout (RLA5086)

Rainbow trout farming is one of the major aquaculture activities in Chile. Infectious pancreatic necrosis (IPN) is a viral disease with considerable negative impact on the rainbow trout (Oncorhynchus mykiss) aquaculture industry. Despite the availability of vaccines against the IPNV, the protective efficacy is relatively low. Breeding rainbow trout for enhanced resistance against IPNV is one of the long-term strategies for controlling the disease. Such breeding approaches are being attempted by commercial rainbow trout breeding companies following conventional selective breeding techniques. Application of advanced technologies such as genomic selection will help improve the genetic gain at a relatively faster rate. In this regard, Facultad de Ciancias, Veterinarias y Pecuaria of Universidad de Chile, Santiago, Chile initiated a genome wide association study on resistance to IPNV. A total of 1093 trout (including vaccinated and unvaccinated) were challenged with IPNV, and the phenotypes were recorded as mortality/alive, survival time, and viral load characteristics. All the trout were genotyped using Axiom™ Trout Genotyping Array that consisted of 57501 markers spaced across the genome (including 17000 markers discovered in American trout and 20000 markers derived from Norwegian trout). Of which, 1032 samples were genotyped successfully with the extraction of information on 36084 SNPs (62.75%). The pruning of genotype data and the analysis to perform genotype-phenotype associations is currently under progress.

Marker Metrics Summary

- Number of Markers: 57501
- Number of BestandRecommended: 36084
- Percent BestandRecommended: 62.754

ConversionType	Count	Percentage
PolyHighResolution	28852	50.177
CallRateBelowThreshold	11421	19.862
Other	9703	16.874
NoMinorHom	4448	7.736
MonoHighResolution	2784	4.842
OTV	293	0.51



Rainbow trout genotyping using Axiom array

Antimicrobial Resistance in Animal Production Environments: Preliminary Analysis of Bioaerosol, Faeces, and Wastewater

Antimicrobial resistance (AMR) in bioaerosols present in animal farms is a growing concern due to its potential implications for animal and human health. Bioaerosols, which are airborne particles containing microorganisms, can act as vehicles for the transmission of antibiotic-resistant bacteria. Understanding the dynamics and occurrence of AMR in bioaerosols within animal farm settings is crucial for developing effective strategies to mitigate the spread of AMR at the animal-environment-human interface.

Building on the Animal Production and Health Laboratory's (APHL) ongoing collaborations with various partners, the study endeavors to investigate the bacterial communities and AMR within the air, faeces, and wastewater found in animal production environments. Samples collected using six-stage Andersen air samplers were analysed with antimicrobial susceptibility testing (AST) and molecular methods. A total of 81 colonies randomly selected among the acquired bacterial communities derived from the bioaerosols of pig and cattle farms were analysed. Figure 1 presents the relative abundance of different bacterial genera cultured from bioaerosols collected at pig and cattle farms. Notably, Staphylococcus is prevalent in both environments, with a higher proportion found in the cattle farm bioaerosols (27.03%) as compared to the pig farm (18.18%). In contrast, Rothia is the dominant species in the pig farm samples, constituting 25% of the bacteria found, but it is not present in the cattle farm samples. Pseudomonas and Bacillus are common to both farm types but are more abundant in the cattle farm samples. The presence of specific bacteria in the bioaerosols could be influenced by the type of livestock, the farming practices, and other environmental factors.

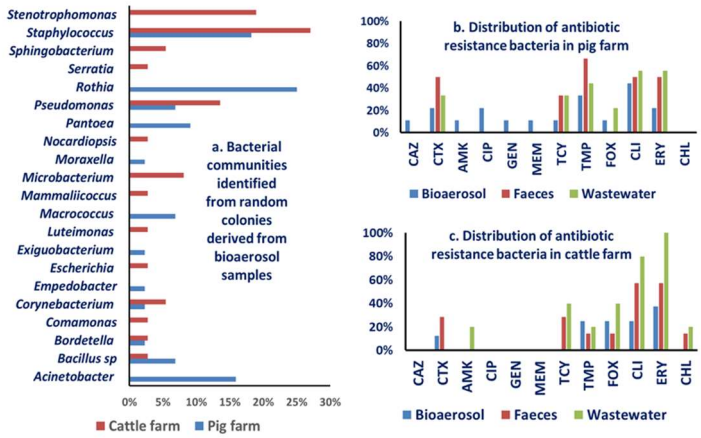


Figure 1. (a) Genus of randomly selected bacterial colonies present in bioaerosol samples collected from pig and cattle farms; Distribution of antibiotic resistance bacteria in bioaerosols, faeces, and wastewater in (b) pig farm and (c) cattle farm

An investigation was also conducted on 24 gram-negative bacteria from the pig farm and 20 gram-negative bacteria from the cattle farm to explore AMR patterns. The resistance patterns in bioaerosols, faeces, and wastewater samples showed a common trend of susceptibility to most antibiotics. However, faecal samples displayed a higher incidence of resistance, while bioaerosols from the pig farm exhibited a more diverse resistance pattern. The preliminary results suggest that bioaerosols may play a significant role in AMR transmission in animal farm environments. Further sampling, analysis, and statistical testing are currently in progress.

Animal Health

Cultivation and Expansion of Genotype I African Swine Fever Virus Strains for Vaccine Development – The Irradiated ASFV Vaccine Project

African swine fever virus (ASFV) is among the most devastating and economically significant diseases affecting the pig industry. To date, there is no licensed vaccine for ASFV, and current control methods involve biosecurity measures and culling of animals in affected areas and regions. ASFV can cause high morbidity and mortality in domestic pigs and wild boars. When introduced into disease-free regions in domestic pig populations, the disease predominantly shows acute forms with high mortality rates up to 100%. After several years of ASFV presence in endemic areas, mortality rates declined due to virus adaptation to the hosts, and infected individuals show subacute forms of the disease or even no clinical signs, complicating even more its detection and eradication.

Currently, there are 24 genotypes of ASFV based on the major capsid protein p72, and 8 serotypes based on the viral hemagglutinin CD2-like protein (CD2v) and C-type lectin. Genotypes I and II are considered "global" as they have spread out of Africa in the past decades.

ASFV is stable in the environment and can be readily transmitted through infected pork products and contaminated fomites. Thus, ASFV poses a significant threat to the swine industry worldwide, and the need for an ASFV vaccine is of high priority. The pursuit for an effective vaccine against ASFV has been largely unsuccessful.

Different vaccine strategies for ASF have been evaluated in the past decades: inactivated vaccines, DNA vaccines, subunit vaccines, and adenovirus-vectored vaccines have been tested and proved to be unsuccessful. Some gene-deleted ASFVs have shown concrete potential as live attenuated vaccines even though their applicability in the field was always mined by safety concerns, as previous studies showed their capacity to convert to a more virulent strain during replication in host animals.

That is why a completely inactivated ASFV vaccine should be considered the safest and the most reliable option, worth research investment. So far, ASFV vaccines inactivated by classic methods (chemical or heat inactivation) have been unsuccessful. They were very efficient at inducing antibodies, on occasion capable of blocking the virus in fluids, but not at inducing specific cytotoxic CD8+ T cells (CTLs), pivotal for elimination of virus-infected cells.

Despite these discouraging premises, gamma-irradiation can represent a powerful and resourceful technology to inactivate ASFV and develop a potential vaccine candidate, due to its ability to preserve the outer structure of the virus, therefore its antigenicity and immunogenicity. This is why scientists at APHL are currently growing isolates of ASFV Genotype I from Cameroon in the BSL3 containment, located in the Austrian Agency for Health and Food Safety (AGES). The current 'Irradiated ASFV Vaccine Project' was designed and conceptualized together with the National Veterinary Laboratory (LANAVET) in Cameroon, which also provided the ASFV Genotype I isolates, and it foresees different phases:

1. Selection of one Genotype I ASFV strain from Cameroon showing clear cytopathic effect (CPE) and hemadsorption (HAD).
2. Growth and expansion of the selected virus strains in primary cells (macrophages) or established cell lines (ZMAC4 and IPKM) targeting a high viral titer.
3. Inactivation by gamma-irradiation of the viral batch.
4. Conduct an immunization clinical trial in swine in LANAVET, Cameroon, testing different inoculation methods (intradermal and oromucosal) and different vaccine compositions.
5. Analyze tissue, sera, and blood samples from immunized animals to identify potential correlates of protection.

The outcome of this study will inform on what is the most promising vaccine composition and inoculation route to be tested in the next stage of the clinical trial - including immunization and challenge of the animals.

Currently, "phase 2" is about to be completed, as the selected strain has been identified and the target titer almost reached. This identified strain was demonstrated to be suited for the hemadsorption assay (HAD) (Fig.2a,2b), and it shows clear CPE in cells. HAD represents the standard method for diagnosis and for virus titration of (hemadsorbing) ASFV isolates; its basic principle is that swine macrophages, once infected with ASFV, display on their surface the viral CD2v protein, which is able to bind erythrocytes. When red blood cells bind around the surface of an infected macrophage, it is possible to observe the classic "rosette" shape (Fig. 2c) indicating a clear sign of viral infection. With microscopic observation of the rosettes at 48, 72 and 120 h.p.i (hours-post-infection) it was possible to confirm the infection and to calculate viral titer.

The next phase of the project will see the inactivation by gamma radiation at the laboratories of the Joint FAO/IAEA Centre.

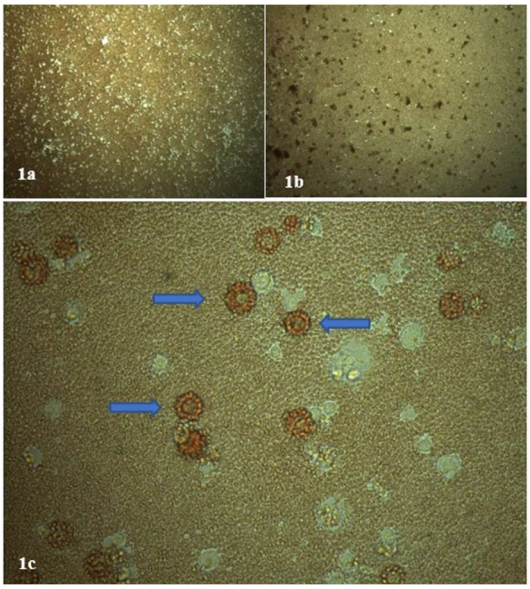
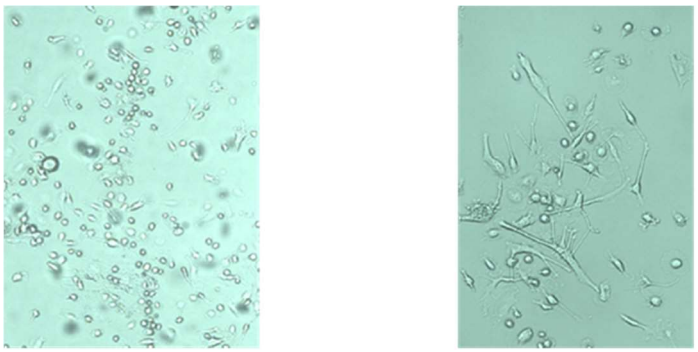


Figure 2: HAD of Genotype I ASFV strain – pictures taken during an IAEA training delivered to AGES staff - (2a) HAD- Negative Control; (2b) HAD+ ASFV infection of macrophages; 2c) Zoom in and clear visualization of "rosette"-like shape due to the disposition of erythrocytes around infected macrophages.

Development of an Immortalized Porcine Monocyte Derived Dendritic Cell Line using Irradiation

Dendritic cells are essential antigen presenting cells of the immune system and are responsible for priming naïve T cell populations in lymph nodes after encountering antigen in periphery tissue, thereby linking innate to adaptive immunity. In pigs, dendritic cells are susceptible to various viruses including African swine fever virus (ASFV), classical swine fever virus (CSVF), porcine circovirus 2 (PCV2), porcine reproductive and respiratory syndrome virus (PRRSV), and swine influenza virus (SwIV). Monocyte derived dendritic cells (MoDCs) can be generated *in vitro* by culturing monocytes in medium that is supplemented with recombinant GM-CSF (granulocyte macrophage colony-stimulating factor) and IL-4 (interleukin 4). Radiation experiments in conjunction with CRISPR, a gene editing tool that was used to inactivate the p53 gene that is responsible for initiating programmed cell death, were used together to attempt immortalising lab generated swine MoDCs. Briefly, PBMCs (peripheral blood mononuclear cells) were isolated from swine whole blood using density gradient centrifugation before purifying CDl4+ cells (Monocytes) with CD14+ magnetic beads. Isolated monocytes were then electroporated with a CRISPR RNP (ribonucleoprotein) complex targeting p53 in 3 places. Transfected cells were then irradiated every other day for 2 weeks and are currently under observation for transformation. If successful, the MoDC cell line generated will be useful for in vitro viral replication and shared with member state laboratories to carry out *in vitro* antigen presentation experiments for porcine vaccines.

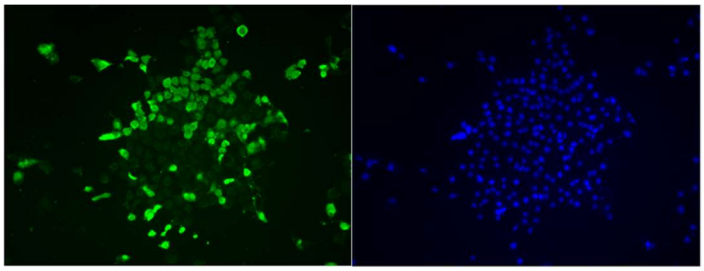


Left isolated monocytes right; MoDC

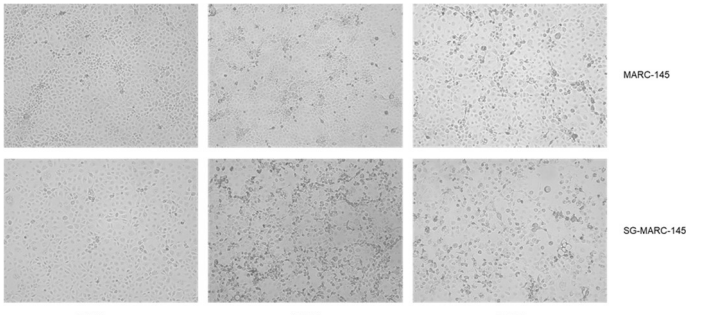
Porcine Reproductive and Respiratory Syndrome (PRRS)

Porcine Reproductive and Respiratory Syndrome (PRRS) poses a significant threat to the robust development of the swine industry, resulting in substantial economic losses annually. The causative agent of PRRS is the Porcine Reproductive and Respiratory Syndrome Virus (PRRSV). This viral infection induces reproductive disorders in maternal sows during the late gestation period, leading to complications such as preterm labour, abortion, or stillbirth. Additionally, PRRSV manifests as respiratory symptoms in new-born piglets.

Presently, two commercial PRRS vaccines are available: a modified attenuated vaccine and an inactivated vaccine. While the attenuated vaccine is acknowledged for its effective protection, concerns persist regarding its cross-protection capability and safety. Conversely, the inactivated vaccine, despite its good safety profile, exhibits limited efficacy.



MACR-145 infected with PRRSV



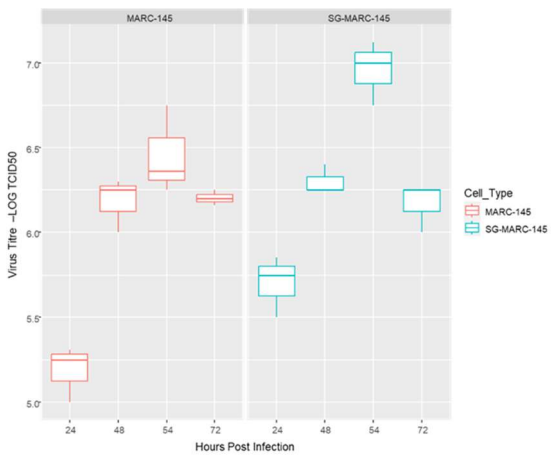
CPE observed microscopically at 24, 48, and 72 HPI

In recent years, Southeast Asian countries, including China, Thailand, Vietnam, and Cambodia, have witnessed a significant challenge posed by highly pathogenic Porcine Reproductive and Respiratory Syndrome (HP-PRRS) attributed to mutated strains of the PRRSV, commonly

known as the blue ear disease virus. This outbreak has resulted in exceptionally high mortality rates, impacting swine of nearly all age groups. In response to the pressing demand for a vaccine characterized by both safety and efficacy, we initiated collaborative research with China Agricultural University, focusing on irradiated vaccines.

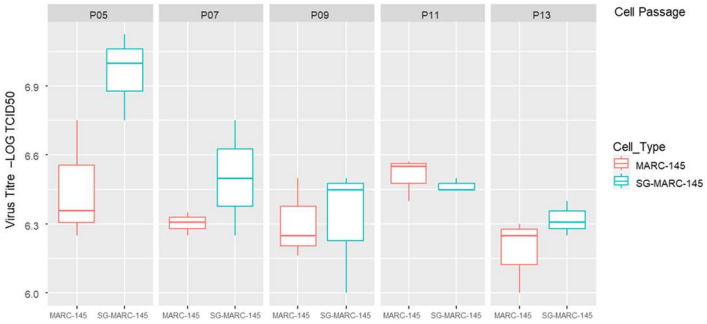
A strain of Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) was isolated from the field, and its genotype was identified as PRRSV-2 (previously genotype 2, Type 2 or North American – NA). Subsequently, we cultured this virus in the laboratory using MARC-145 cells. The Immunofluorescence assay (IFA) was conducted, and the PRRSV virus particle was stained green. We modified the MARC-145 by transfecting swine Granulocyte-macrophage colony stimulating factor (GM-CSF). Cytopathic effect (CPE) in MARC-145 and SG-MARC cell culture infected with PRRSV was observed microscopically at 24, 48, and 72 hours post infection (HPI).

Viral growth curve analysis was performed in both the MARC-145 cell line and SG-MARC-145 cell line. Cell monolayers of the two cell lines in T25 flask were infected with PRRSV. After 1 h of incubation at 37°C in 5% CO2 with shaking every 15 minutes, the inocula were removed, and the monolayer was washed three times with serum-free DMEM. After washing, 5 ml 2% FBS DMEM was added, and the flasks were then incubated for up to 72 hours at 37°C in 5% CO2. Supernatants were collected at 24, 48, 54, and 72 hours post infection (HPI) and titrated by the method of Reed-Muench on Marc-145 cells in 96-well plates. The percentage of wells positive for CPE was calculated and recorded as TCID50/ml. Based on the TCID50 results, the maximum viral titre was observed at 54 HPI in both cell lines. We investigated whether the transfected cell lines SG-MARC-145 could enhance viral replication. Virus infection experiments were conducted on both transfected and normal cell lines at the 5th, 7th, 9th, 11th, and 13th passages, respectively. Viruses were harvested 54 hours post infection, and their titres were determined using the Reed-Muench method on MARC-145 cells in 96-well plates. The



Viral growth curve of PRRSV in MACR-145 and SG-MARC-145 cell line

results indicated that, in the early passages of the transfected cell line, the viral titre was indeed higher compared to that of the normal cell line, suggesting a potential contribution of the secretion of swine GM-CSF to viral enhancement. However, this trend gradually weakened with an increase in the number of passages, possibly due to insufficient stability of the transfected cell line. In subsequent experiments, we aim to monitor the expression level of the transfected gene GM-CSF and explore strategies to enhance the stability of the transfected cell lines.



Comparison of viral replication in different cell lines

Detection of Porcine Circovirus 3 (PCV-3) in Namibian Backyard Farms and Warthogs

Since its first identification in 2015, porcine circovirus 3 (PCV-3) has been reported worldwide with a high frequency and in the presence of several clinical conditions, although its impact on pig health and productivity is still debated. Data on PCV-3 presence in Africa are, on the other hand, limited. A previous study performed on Namibian commercial pigs failed to identify the pathogen. In the present study, the viral circulation in backyard farms, characterised by lower biosecurity measures and frequent animal exchange between farms, was assessed. The susceptibility of warthogs to PCV-3 infection and their potential epidemiological role were also evaluated. Tonsils from 77 pigs from backyard piggeries and 55 warthogs were collected in different regions of Namibia and tested by PCR. Positive samples were sequenced and compared to PCV-3 strains circulating globally. Overall, 42 out of 77 pigs (54.54%) and 12 out of 55 warthogs (21.82%) tested positive, demonstrating the presence of PCV-3 in the country and suggesting that the high biosecurity measures implemented in commercial farms probably prevent viral introduction. The partial ORF2 gene was successfully sequenced in samples from 27 pigs and 6 warthogs. Genetically, the identified strains were part of 3 distinct groups which included both backyard pigs and warthogs from different Namibian regions. There is also evidence for the occurrence of multiple introduction events most likely from Asian countries, either directly or through other African countries.

First Detection and Molecular Characterization of Porcine Reproductive and Respiratory Virus (PRRSV) in Namibia

Porcine reproductive and respiratory syndrome (PRRS) is among the most important infectious diseases affecting swine worldwide, but information on its epidemiology in Africa is extremely limited. In the present study, 147 healthy butchered pigs, originating from 15 Namibian intensive and rural farms were tested by RT-PCR, and the ORF7 genes of positive samples were sequenced for further genetic characterization and phylogenetic analysis. Additionally, 55 warthogs were also evaluated using the same approach. Overall, 7 out of 147 pigs (4.76%) tested positive, all originating from 3 rural farms. All intensively-farmed pigs and warthog samples were negative. Sequence analysis revealed that all strains belonged to the Betaarterivirus suid1 species (previously known as PRRSV type I) and were likely imported from Europe at least 6 years ago, evolving independently thereafter. Based on the findings, the presence of the PPRSV appears limited to backyard farms. While biosecurity measures applied in industrial farms appear to be effective in preventing viral introduction, PRRSV circulation in rural settings still represents a potential threat.

Detection of Porcine Circovirus 2 (PCV-2) in Namibian Dog Blood

Porcine circovirus 2 (PCV-2) is a recognized main pathogen of swine, causing remarkable losses worldwide. However, several studies have shown that it can also be detected in other species. The present study tested more than 500 archived blood samples of Namibian dogs for the presence of PCV-2 DNA. Overall, 38 out of 575 blood samples (6.61%) tested PCV-2 positive, and the ORF2 of 7 strains was sequenced identifying 3 of the major PCV-2 genotypes (i.e. PCV-2a, -2b, and 2d). Likely, epidemiological links with other Namibian and South African strains were established for PCV-2a and PCV-2b strains, while the PCV-2d ones were part of a broader clade including worldwide collected sequences, especially from Asia. The infection of Namibian dogs is believed to be most likely due to the ingestion of contaminated meat and byproducts.

Bovine Coronavirus Presence in Domestic Bovine and Antelopes Sub-Saharan Africa: Evidence from Namibia

Bovine coronavirus (BoCV) causes significant economic losses due to mortality in calves, as well as reduced growth performance and milk production in adult feedlots and dairy cattle, respectively. Worldwide distribution of BoCV has been demonstrated, although knowledge of its epidemiology in Africa, especially in the sub-Saharan region, is limited. A total of 208 swab samples of wild ruminants and 435 bovines from different regions of Namibia were obtained and tested using a BoCV-specific qRT-PCR. Twenty-six bovine samples and one Greater kudu (*Tragelaphus strepsiceros*) sample were shown to be positive. Analysis of partial nucleoprotein and spike protein gene sequences, as well as comparison with international reference sequences, demonstrated the existence of a unique Namibian clade, resulting from a single introduction event around 2010 followed by local evolution. The implications for disease spread among domestic bovines and the potential impact on wildlife should encourage broader investigations on BoCV involving other African countries.

Molecular Characterization of Rabies Viruses in Nepal from Different Hosts

In September 2023, RNA extracted from brain samples collected from dogs (n=4), buffalo (n=2), cattle (n=3) and goats (n=3) were provided by the Central Veterinary Laboratory in Katmandu, Nepal. The RNA was tested for the presence of the nucleoprotein (N) and glycoprotein (G) genes using conventional RT-PCR. N gene amplicons obtained from eight of the samples were purified and sequenced. A neighbour-joining phylogenetic tree with 1000 bootstraps was performed using the obtained sequences and others available in GenBank (Figure 3). The phylogenetic tree shows that all the sequences from the current samples belong to artic-related clade 1a, as previously identified in Nepal.

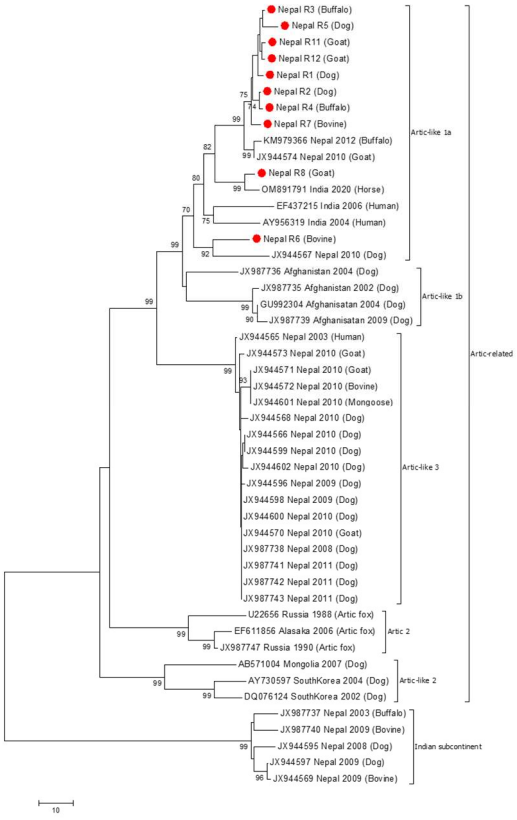


Figure 3: Phylogenetic tree generated using the RABV sequences from Nepal

The Evaluation of Five Serological Assays in Determining Seroconversion to Peste des Petits Ruminants Virus in Typical and Atypical Hosts

A collaborative work carried out by the Animal Production and Health Laboratory together with The Pirbright Institute, University of Glasgow Centre for Virus Research, Tanzania Wildlife Research Institute (TAWIRI), Pan African Veterinary Vaccine Centre for African Union, and Royal Veterinary College (RVC), London resulted in a recent publication in the journal Scientific Reports. The work was based on detection of antibodies against peste des petits ruminants (PPR). PPR is an infectious viral disease of small ruminants which also infects a wide range of wild and domestic animals such as African buffalo, gazelle, saiga, and camels. The role of wildlife as a reservoir of the disease is poorly understood. Assays such as virus neutralisation test (VNT) and enzyme-linked immunosorbent assay (ELISA), are serological methods that have been validated using sera from domestic and typical host species. The ability of these assays in detecting antibodies from wildlife and atypical species is not clear.

A comparative analysis of VNT, ID VET N-ELISA, and AU-PANVAC H-ELISA results from a large panel of sera (n = 793) from a range of species from multiple countries (sourced 2015-2022) were compared. A sub-panel (n = 30) was also distributed to two laboratories and tested using the luciferase immunoprecipitation system (LIPS) and a pseudotyped virus neutralisation assay (PVNA). A 75.0-88.0% agreement of positive results was shown for detecting PPRV antibodies in sera from typical species between the VNT and commercial ELISAs; however, this decreased to 44.4-62.3% in sera from atypical species, with an inter-species variation. The LIPS and PVNA strongly correlate with the VNT and ELISAs for typical species but vary when testing sera from atypical species.

Harnessing Attenuation-related Mutations of Viral Genomes: Development of a Serological Assay to Differentiate between Capripoxvirus Infected and Vaccinated Animals

The article entitled "Harnessing Attenuation-related Mutations of Viral Genomes: Development of a Serological Assay to Differentiate between Capripoxvirus Infected and Vaccinated Animals" was published in November 2023 in the journal Viruses. The need for a test capable of differentiating between infected and vaccinated animals (DIVA) was described by many experts as one of the most important tools to establish proper disease surveillance, control, and eradication programmes.

While comparing the full genome sequences of the live-attenuated Capripox vaccines and field isolates, we observed that many genes, including some that encoded for proteins involved in immune evasion were truncated in the sheeppox vaccine viruses and not in the field isolates of sheeppox virus. A similar analysis using lumpy skin disease virus (LSDV) showed only a few genomic changes (point mutations) between vaccine strains and LSDV field isolates. When amino acid sequence analysis was performed on all three species of Capripoxviruses, it became evident that these mutations in the vaccine strains led to truncation in a number of genes, including the B22R. We designed a peptide downstream from the mutations to target specifically the field strain of Capripoxvirus and not the vaccine strains. Using our experience in developing the A34 iELISA, we tested well-characterized infected and vaccinated sera. We developed the wildtype-specific Capripox DIVA iELISA, which showed >99% sensitivity and specificity for serum collected from animals infected with wildtype virus. The test showed no cross-reactivity to anti-parapoxvirus antibodies and could detect seroconversion in cattle between 8 and 12 DPI up to the last collection at 30 DPI. In goat samples, the test detected seroconversion between 7 and 14 DPI up to at least 49 DPI.

Together with the previously described A34 iELISA, which can detect vaccinated and infected animals, these two ELISAs could be the basis for an eradication programme for Capripoxviruses since they can paint a clear picture of the serological status of individuals and populations in a country or region. These assays could determine freedom with vaccine and freedom from disease status. To our knowledge, the DIVA iELISA for Capripox is the first ever poxvirus DIVA assay based on naturally occurring genetic changes consequence of vaccine attenuation. This assay works with established and commonly used Capripox vaccines, so it does not require the deployment of new vaccines, saving costs in its implementation.

Comparison of the Whole Genome Sequence of African Swine Fever Virus from a Mongolian Wild Boar with Genotype II Viruses from Asia and Europe

African swine fever virus (ASFV), characterized by its pronounced lethality but constrained transmission dynamics, emerged in the Caucasus region in 2007. Despite its limited contagiousness, ASFV has presented substantial epidemiological challenges, significantly affecting both domestic and wild *Sus scrofa* populations throughout Europe and Asia.

This study details the whole genome sequencing of the ASFV from a wild boar in Mongolia and compares it with existing Asian and European sequences. GrapeTree visualization (Figure 1) delineates European and Asian ASFV sequences into two distinct clusters. The Mongolian

ASFV genotype II is largely congruent with Asian genotypes but exhibits three nucleotide differences from European strains, two resulting in non-synonymous mutations within the MGF360 and ACD 00190 genes. Despite the 409 nucleotide substitutions between the continents' sequences, an overall genomic homogeneity exceeding 99.99% persists. The diversity within European ASFVs is suggested to stem from a broader temporal range of sequences (2007-2022) relative to the Asian samples (post-2018). The study underscores the slow mutation rate of ASFV and the close genetic relationship between the virus in wild boars and domestic pigs, implicating potential direct or indirect transmission routes.

In figure 4, a GrapeTree visualization of 27 WGS shows that the European and Asian sequences are separated into two distinct clusters, with the European sequences (blue) being more dispersed than the Asian sequences (green).

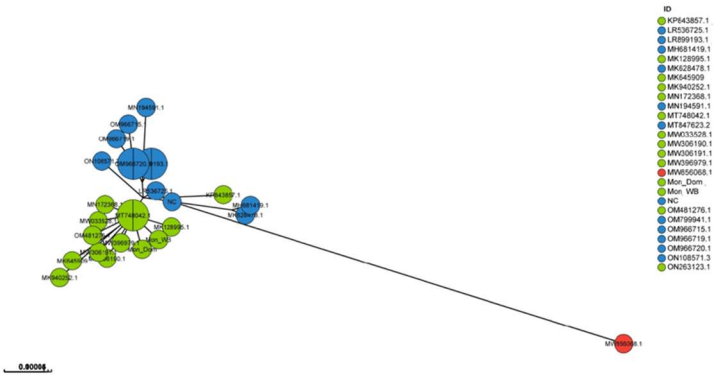


Figure 4: GrapeTree interface exemplified with a precalculated Newick tree based on 27 WGS of ASFV genotype II. Nodes in blue include European sequences, nodes in green show Asian sequences, and Africa is shown as red.

Capacity Building

FAO Emergency Stockpile: Shielding Health and Livelihoods from Animal Disease Outbreaks

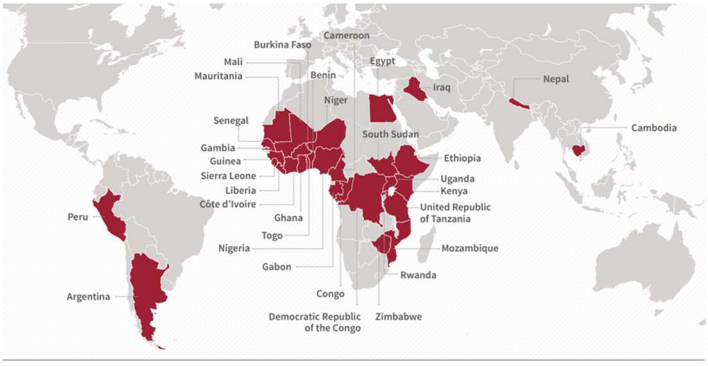
Agathe Auer

The Food and Agriculture Organization of the United Nations (FAO), with funding from the United States Agency for International Development (USAID), is managing the Global Stockpile for Emergency Animal Diseases. This initiative, part of the Emergency Centre for Transboundary Animal Diseases (ECTAD), is key for timely responses to zoonotic disease outbreaks and supports global health security. It provides rapid access to diagnostic reagents, personal protective equipment (PPE), and laboratory consumables, essential for early outbreak detection and response, particularly where local procurement is challenging. Stocks are maintained at the FAO headquarters in Rome, Italy, the Joint FAO/IAEA Centre for Nuclear Techniques in Food and Agriculture's Animal Production and Health Laboratory (APHL) in Seibersdorf, Austria, and the United Nations Humanitarian Response Depot in Dubai, United Arab Emirates. The FAO/IAEA APHL has diagnostic reagents ready for dispatch for ten outbreaks, with capacity for 1,000 tests per outbreak, and regularly rotates these stocks. Laboratory consumables are also stored in adequate quantities in Rome.

Additionally, the FAO facilitates training on the shipment of infectious substances, ensuring countries can comply with the International Air Transport Association (IATA) Dangerous Goods Regulations (DGR), and coordinates shipments to FAO reference laboratories for diagnosis and molecular analysis. Where permitted, genetic sequences from these activities are made openly available.

By providing these services, FAO enables governments to enact swift disease control measures, thus protecting the health of people, animals, and the environment.

Figure 1. Countries supported by the Global Stockpile (for the period of 1 January 2015 to 31 July 2023)



Source: **United Nations Geospatial.** 2023. *Map of the World.* [Cited 4 September 2023]. https://www.un.org/geospatial/content/map-world-1 The final boundary between the Sudan and South Sudan has not yet been determined. Final status of the Abyei area is not yet determined. The dotted line represents, approximately, the Line of Control in Jammu and Kashmir agreed upon by India and Pakistan. The final status of Jammu and Kashmir has not yet been agreed upon by the parties.

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Fellows, Interns, Consultants

Mr Federico Verly joined the Joint FAO/IAEA Centre, Animal Production & Health Section (APH) as a consultant to support the implementation of iVetNet and the ZODIAC initiative on 1 October 2023. Federico's main responsibilities are related to the development and finalisation of the mobile application for upload of equipment and room monitoring records on iVetNet, the update and upgrade of iVetNet/ZODIAC database/bioinformatic modules, the provision of training and guidance to operate the database platform and modules, the update of inventory data, including records on certification and calibration of instruments/pipettes and the preparation of information reports, compilation of documents and procedures and provision of logistical backstopping to ZODIAC events, capacity building and transfer of technologies.

Mr David Alejandro Tapio Espinoza from Aquaculture Genomics Laboratory, Universidad de Chile, Santiago, Chile was trained at APHL, Seibersdorf on "Genome-wide typing of rainbow trout for association with resistance to infectious pancreatic necrosis virus" for two months (18 September to 17 November 2023) under TC fellowship (FS-RLA5086-2301917-001).

Ms Carolina Andrea Araya Pulgar from Facultad de Ciancias, Veterinarias y Pecuarias, Universidad de Chile, Santiago, Chile was trained at APHL, Seibersdorf on "Genome-wide typing of rainbow trout for association with resistance to infectious pancreatic necrosis virus" for two months (18 September to 17 November 2023) under TC fellowship (FS-RLA5086-2301917-002).

Mr Adhemir Ayrton Valera Andrade from Universidad Nacional Mayor de San Marcos, Lima Peru was trained at APHL, Seibersdorf on "Genome-wide typing of rainbow trout for association with resistance to infectious pancreatic necrosis virus" for two months (18 September to 17 November 2023) under TC fellowship (FS-RLA5086-2301917-003).

Mr Menghak Phem from General Directorate for Animal Production and Health, Ministry of Agriculture, Forestry and Fisheries, Phnom Penh, Cambodia was trained at APHL, Seibersdorf on "Industrial production and quality control of frozen semen for cattle breeding" for six weeks (13 November to 22 December 2023) under TC fellowship (FS-KAM5009-2304312).

Ms Mariana Paz Calderon Nieto from Haciendo San Juan Molino Carretera Estatal Tecuxcomac, Tlaxcala, Mexico was trained at APHL, Seibersdorf on "Molecular techniques for characterization of livestock" for two months (2 October to 30 November 2023) under TC fellowship (FS-MEX5033-2302016).

Ms Tafara Kundai Mavunga from Zimbabwe is undergoing her internship training at APHL, Seibersdorf on "Genomic characterization of indigenous livestock breeds" for one year from 3 July 2023 to 28 June 2024.

Ms Sonja Allen from Jamaica was trained as an intern at APHL, Seibersdorf on "Genomic analysis of Eastern European cattle and cryopreservation of gametes" for 3.5 months from 17 July to 28 October 2023.

Coordinated Research Projects (CRPs)

Project Number	Title	Project Officers
D31030	Improving Efficiency of Animal Breeding Programs Using Nuclear Related Genomic Information – Practical Applications in Developing Countries	V. Tsuma K. Periasamy
D31031	Nuclear and Related Techniques to Measure the Impact of Type of Feeding and Production System on Greenhouse Gas (GHG) Emissions and Livestock Productivity	V. Tsuma
D32034	Use of Stable Isotopes to Trace Bird Migrations and Molecular Nuclear Techniques to Investigate the Epidemiology and Ecology of the Highly Pathogenic Avian Influenza - Phase II	I. Naletoski
D32035	Improvement of Diagnostic and Vaccine Tools for Emerging and Re-emerging Animal Health Threats	C. Bravo de Rueda V. Wijewardana
D32036	Application of Advanced Molecular Characterization Technologies Through the Veterinary Diagnostic Laboratory Network (VETLAB Network)	I. Naletoski
D32037	Novel Test Approaches to Determine Efficacy and Potency of Irradiated and Other Vaccines	V. Wijewardana C. Bravo de Rueda
D32038	Enhancing laboratory preparedness for the detection and control of emerging and re-emerging zoonotic diseases – ZODIAC in the Americas and the Caribbean	C. Lamien
D32039	Enhancing laboratory preparedness for the detection and control of emerging and re-emerging zoonotic diseases – ZODIAC in Asia and the Pacific	C. Lamien
D32040	Enhancing laboratory preparedness for the detection and control of emerging and re-emerging zoonotic diseases – ZODIAC in Europe and Central Asia	C. Lamien
D32041	Enhancing laboratory preparedness for the detection and control of emerging and re-emerging zoonotic diseases – ZODIAC in Africa	C. Lamien
D32043	Innovative Nuclear and Related Molecular Approaches for Detection and Characterization of Antimicrobial Resistance in Animal Production Environment	K. Periasamy

Submission of Proposals

Research contract proposal forms can be obtained from IAEA, the National Atomic Energy Commissions, UNDP offices or by contacting a Project Officer. The form can also be downloaded from http://cra.iaea.org/cra/index.html

Improving Efficiency of Animal Breeding Programs Using Nuclear Related Genomic Information – Practical Applications in Developing Countries (D31030)

Victor Tsuma and Kathiravan Periasamy

The Coordinated Research Project (CRP) aims to enable use of nuclear and related genomic technologies in Member States to enhance the efficiency of national breeding programmes for increased milk productivity and dairy animal adaptability to the production environment. Specifically, the CRP aims to a) develop nuclear and related genomic tools/resources such as radiation hybrid maps and DNA microarrays for tropical dairy species, and b) identify genomic regions of importance for milk and adaptability traits in local dairy animal populations, c) establish strategies to incorporate genomic information for selection and breeding of dairy animals, and d) develop and validate radiolabelled biomarker assays for early pregnancy diagnosis in cattle. Three major dairy animal species viz. cattle, buffalo and camel have been targeted. Eleven

research contracts awarded to institutes in 10 developing countries from Africa, Asia and Latin America are progressing with year two project activities, having successfully implemented those of year one.

Nuclear and Related Techniques to Measure the Impact of Type of Feeding and Production System on Greenhouse Emissions and Livestock Productivity (D31031)

Victor Tsuma

This Coordinated Research Project (CRP) aims to enable the Member States (MS) of the IAEA, particularly in developing countries, to use nuclear and related technologies and resources to optimize livestock feeding practices that reduce greenhouse gas (GHG) emissions and help mitigate climate change. Specifically, the CRP aims to a) evaluate nitrogen and energy supplementation strategies in cattle feeding to mitigate enteric and manure GHG emission, b) to develop and/or validate nuclear and related tools/resources for nutrition related GHG mitigation in cattle production, and c) to provide MS with tools and mechanisms to monitor livestock GHG emissions. Targeted are dairy cattle production systems. The 10 research contracts awarded to institutes in 10 developing countries from Africa, Asia and Latin America successfully carried out year one project activities and are now implementing the second year work plans

Use of Stable Isotopes to Trace Bird Migrations and Molecular Nuclear Techniques to Investigate the Epidemiology and Ecology of the Highly Pathogenic Avian Influenza Phase II (D32034)

Ivancho Naletoski

The aim of this Coordinated Research Project (CRP) is to evaluate the origin of wild birds that carry Avian Influenza (AI) and other potentially dangerous pathogens at their stopover places and match the obtained results with the knowledge obtained through conventional migration monitoring approaches.

Stable isotopes (SI) are promising huge potential when the origin (migration) of individual wild birds is required, because the probability of capturing a labelled bird with specific characteristics (disease carrier) using conventional methods is negligible.

Knowledge and experience obtained through the previous project (D32030 - Use of Stable Isotopes to Trace Bird Migrations and Molecular Nuclear Techniques to Investigate the Epidemiology and Ecology of the Highly Pathogenic Avian Influenza) will be of great value for the success of this project.

The use of SI in migration studies of wild animals, including wild birds, primarily in environmental protection studies and conservation activities, has attracted the attention of the scientific community; however, this technique can also be used in epidemiological studies that target long-range transmission of animal pathogens.

The development and maintenance of the IAEA Global Network of Isotopes in Precipitation (GNIP) became a significant facilitator of these studies, as it offered geo-spatial reference values for correlation of the SI ratios in the animal tissues (especially metabolically inert tissues like beaks, claws and feathers) and the SI ratios in the environment (especially open waters).

During the first phase of this CRP, several important steps in the linking of SI ratios of feather samples (bird migrations) with the epidemiology of AI were established. Achievements of project D32030 have shown not only that the isotope assignment works, but also have delivered a full package of techniques that will strengthen and supplement (SI component) the official wild bird monitoring programmes of Member States.

In the current project, the partners will focus on two critical issues:

a) detecting birds that carry avian influenza viruses and eventually other dangerous pathogens, and

b) evaluating stable isotope ratios in feathers of these birds (only the pathogen carriers) to understand their origins and migration pathways.

Improvement of Diagnostic and Vaccine Tools for Emerging and Re-emerging Animal Health Threats (D32035)

Carla Bravo de Rueda and Viskam Wijewardana

Vaccination has proven to be the best preventive measure against infectious diseases. Despite significant successes, there are several limitations to the currently practiced approaches. In veterinary medicine, the application of vaccines by injection frequently limits their use for small ruminants and poultry. This practice requires well-trained staff taking care to practice the utmost hygiene and maintain vaccine cold chain. Further, also in poultry rearing it is not easy to inject individual birds. In addition to that, injected vaccines rarely induce production of specific mucosal antibodies (IgA) covering the mucosal tissues in the nose, mouth and lungs, which are the primary site of

multiplication for bacteria or viruses before they provoke a systemic infection. Such IgA antibodies can efficiently be induced by 'mucosal' vaccines, i.e. formulations that are applied to the nose, mouth or eyes. These mucosal vaccines, especially eye drop vaccines, have the big advantage in requiring small volumes as the vaccine dose. Therefore, the application can be done by village vaccinators and the cold chain will be relatively easy to maintain.

Recent experiments on formulating such mucosal vaccines have presented a number of challenges: a) low viscosity leading to spills; b) unsuitable components for freeze drying; and c) the process of formulating the components appropriately. Among the latest development of this Coordinated Research Project is the research on Fowl cholera (FC) caused by Pasteurella multocida conducted in Ethiopia. When the irradiated FC vaccine was administered to chickens through intranasal and intraocular routes, a 100% protection was observed, as compared to a much lower rate with intramuscular injection. This work is now published in the major research journal "Frontiers in Immunology". Pakistan has improved their viral vaccine production titres significantly using a Celcradle system and have also shown efficacy of their ocular vaccines by improving its immune response. This work has been published in the 13th International Conference on Goats and the Animal Production and Health symposium. Indonesia has shown progress on the irradiation of the bacteria with maintenance of metabolic activity; in addition, they have further characterized chitosan as a vaccine carrier and immune modulator. Kenya has shown progress on pathogens detection by PCR and has initiated students' programmes; they will soon be starting to test the developed vaccines against New Caste and Gumboro in poultry. Cameroon has also made some advances on the area and is now studying the different routes of vaccines administration against PPR. The next research coordination meeting will be in 2025.

Application of Advanced Molecular Characterization Technologies Through the Veterinary Diagnostic Laboratory Network (VETLAB Network) (D32036)

Ivancho Naletoski and Charles Lamien

The Animal Production and Health Section (APH) of the Joint FAO/IAEA Centre has established a free-of-charge Sanger sequencing service for all counterparts of the subprogramme. So far, over 4000 samples have been submitted for Sanger sequencing by 30 counterpart laboratories (mainly partners in the VETLAB Network) and the results were published in 27 articles in peer reviewed journals.

The APH intends to upgrade this service with additional workflows which should enable counterparts' access to service-based Whole Genome Sequencing (WGS) including the possibility for metagenomic analysis.

Such workflows need to be validated, primarily for biological inactivation of the field samples prior to submission, as well as regarding the quality of the DNA / RNA extracted from the field samples. Additionally, standardized bio-informatic package for processing of the raw data and further phylogenetic analysis needs to be validated and verified for use by the counterpart community. In order to perform these activities, a new Coordinated Research Project (CRP) was developed and approved by the management of IAEA. Priority targets for this CRP will be the established users of the Sanger sequencing service of APH. However, the final objective of the CRP is to further disseminate the validated workflows to the wider counterparts' community.

Novel Test Approaches to Determine Efficacy and Potency of Irradiated and Other Vaccines (D32037)

Viskam Wijewardana and Carla Bravo de Rueda

The objective of this Coordinated Research Project (CRP) is to enhance the assessment efforts of irradiation and other new vaccines, along with the use of cutting-edge techniques, in order to ascertain the immune response and develop immunological instruments for the purposes of quality control and effectiveness. The anticipated results include: a) the development of novel in vitro methods for assessing the effectiveness of vaccines, which will replace or minimise the need for animal challenge trials by utilising in vitro assays that utilise irradiated antigens, b) the assessment of immune marker mRNA qPCR and gene expression assays, c) the utilisation of cytokine protein assays such as ELISPOTS or ELISA, and d) the implementation of cell-based quantification assays that utilise flow cytometry, among others.

In order to comprehend the immune response elicited by the particular vaccination and the fundamental techniques used to assess it, this CRP needs input from each participant. These new processes are anticipated to enhance the ability of vaccine production laboratories to conduct more effective quality control of their products in the future. The use of a more technical approach will enhance the reliability of the outcomes, leading to increased trust. A total of six research contracts have been granted under this CRP, namely in Cameroon, Ethiopia, Indonesia, Iran, Sri Lanka, and Tunisia. Additionally, there have been five agreements made with Ethiopia, the United Kingdom, China, Germany, and Argentina. Furthermore, one technical contract has been issued in Italy. Five research contract holders have identified the vaccine candidate strains, have optimized the

protocols for in-vitro expansion and conducted the preliminary irradiation experiments to identify the inactivation dose.

Enhancing Laboratory Preparedness for the Detection and Control of Emerging and Re-emerging Zoonotic Diseases (D32038, D32039, D32040 & D32041)

Multiple zoonotic diseases have impacted public health, peoples' livelihoods, and the global economy in the last few decades. The COVID-19 pandemic is the most recent severe threat, which will have a long-term and far-reaching influence on the population and economy worldwide.

Surveillance and early detection tools and technologies are the critical links in the chain of disease control. They enable the rapid discovery of source and movement of pathogens as well as analysis, planning, and decision-making through the design and implementation of preventive or control measures.

Nuclear, nuclear-derived and -related techniques are reliable tools that can help scientists to investigate, prevent, detect, and contain outbreaks of zoonotic diseases. In addition, the IAEA has considerable experience in assisting the Member States in building their capacity to detect and characterize pathogens early and diagnose diseases rapidly and accurately. Moreover, the IAEA has developed or contributed to developing early detection and characterization tools, nowadays recognized as international testing standards.

Over the last few decades, technological development has enabled miniaturization and multiplexing of diagnostic assays, thus opening new windows in understanding the ecology and evolution of zoonotic pathogens. Next-generation sequencing, nanopore sequencing, and metagenomics-based approaches will enable novel pathogen characterization and discovery and will help to find potential reservoirs, vectors and additional susceptible hosts for known zoonotic pathogens.

ZODIAC in the Americas and the Caribbean (D32038)

Charles Lamien and Giovanni Cattoli

The ZODIAC CRP for the Americas and the Caribbean aims to develop and validate immunological and molecular tools under Pillar 2 of the ZODIAC project. This CRP will help empowering national and regional disease surveillance programmes in the Americas and the Caribbean to identify potential sources of pathogen spill over to humans and identify emerging- and/or re-emerging pathogens with zoonotic risk.

Priority diseases	Examples	Research areas
Respiratory viruses	*Influenza A, Zoonotic Coronoviruses*	Increased (-targeted) surveillance at the A-H interface; sampling procedures
Arboviruses	Zika, Dengue	Early detection, monitoring and surveillance in the animal reservoirs and vectors, point-of-care testing
Emerging zoonoses	*Hantaviruses, Arenaviruses*	Increased surveillance at the A-H interface; sampling procedures, point-of-care testing
Endemic zoonoses	*Rabies, Brucellosis*	Increased surveillance at the A-H interface; point-of-care testing

TARGETED DISEASES/PATHOGENS

ZODIAC in Asia and the Pacific (D32039)

Charles Lamien and Giovanni Cattoli

The ZODIAC CRP for Asia and the Pacific aims to develop and validate immunological and molecular tools under Pillar 2 of the ZODIAC project. This CRP will help empowering national and regional disease surveillance programmes in Asia and the Pacific to identify potential sources of pathogen spill over to humans and identify emerging- and/or re-emerging pathogens with zoonotic risk.

Priority diseases	Examples	Research areas
Respiratory diseases	*Influenza A viruses, Coronaviruses, Henipaviruses*	Increased (-targeted) surveillance at the A-H interface; sampling procedures
Arboviral diseases	*Tick-borne, mosquito-borne, and those transmitted by Culicoides and sand flies*	Early detection, monitoring and surveillance in the animal reservoirs and vectors, point-of-care testing
Haemorrhagic fevers	*caused by Filoviruses*	Increased surveillance at the A-H interface; sampling procedures, point-of-care testing
Waterborne diseases	*caused by Hepevirus, Leptospira, and others*	
Endemic zoonoses	*Rabies, Brucellosis*	Increased surveillance at the A-H interface; point-of-care testing.

TARGETED DISEASES/PATHOGENS

ZODIAC in Europe and Central Asia (D32040)

Charles Lamien and Giovanni Cattoli

The ZODIAC CRP for Europe and Central Asia aims to develop and validate immunological and molecular tools under Pillar 2 of the ZODIAC project. This CRP will help empowering national and regional disease surveillance programmes in Europe and Central Asia to identify potential sources of pathogen spill over to humans and identify emerging and/or re-emerging pathogens with zoonotic risk.

Priority diseases	Examples	Research areas
Vector-borne diseases	*Tick-borne, mosquito-borne, and those transmitted by Culicoides and sand flies, e.g., Flaviviruses (WNV, TBEV) and Bunyaviruses (Hantaviruses, CCHFV, RVFV)*	Early detection, monitoring and surveillance in animal reservoirs and vectors, point-of-care testing
Respiratory diseases	*Influenza A viruses, zoonotic coronaviruses*	Increased (targeted) surveillance at the vector, animal and human levels, sampling procedures
Foodborne diseases		Increased surveillance at the animal-human interface
Endemic zoonoses	*Rabies, anthrax, Lyme borreliosis, leishmaniosis*	Increased surveillance at the animal-human interface; point-of-care testing.
Endemic zoonoses	*Rabies, Brucellosis*	Increased surveillance at the A-H interface; point-of-care testing.

TARGETED DISEASES/PATHOGENS

ZODIAC in Africa (D32041)

Charles Lamien and Giovanni Cattoli

The ZODIAC CRP for Africa aims to develop and validate immunological and molecular tools under Pillar 2 of the ZODIAC project. This CRP will help empowering national and regional disease surveillance programmes in Africa to identify potential sources of pathogen spill over to humans and identify emerging- and/or re-emerging pathogens with zoonotic risk.

Priority diseases	Examples	Research areas
Haemorrhagic diseases	Lassa fever, Marburg disease, Ebola, Crimean-Congo haemorrhagic fever, Rift valley fever	Early detection, monitoring and surveillance in animal reservoirs and vectors
(Re-) Emerging zoonoses	Zoonotic Coronaviruses, avian influenza, monkeypox	Surveillance and monitoring in animal reservoirs; NGS
Other mosquito-borne diseases	Chikungunya, West Nile, Zika, Dengue, Yellow fever	Differential diagnosis and syndromic surveillance
Rabies		Increased animal surveillance, differential diagnosis, vaccination programmes
Endemic zoonoses	Anthrax, Bovine TB, Brucellosis and zoonotic parasites	Differential diagnosis and syndromic surveillance

TARGETED DISEASES/PATHOGENS

Innovative Nuclear and Related Molecular Approaches for Detection and Characterization of Antimicrobial Resistance in Animal Production Environment (D32043)

Kathiravan Periasamy

Antimicrobial resistance is an important global health concern and is considered to be a pandemic in silence causing more than one million deaths annually. Antimicrobial drugs are used in animals for therapeutic, prophylactic and growth promotion purposes. Emergence and transmission of AMR in animal production systems is a major issue, considering the fact that more than two-thirds of antibiotics sold globally are used on animals. A bulk of this is used as growth promoters for improving production efficiency. Hence, identifying effective alternatives will be an important approach to reduce antimicrobial usage in animal production settings. Further, national AMR surveillance programmes have mostly focused on the detection of AMR in human health and in animals for food safety purposes, but not in animal production facilities. AMR surveillance in animal production settings is constrained by lack of (i) guidelines and harmonized sampling methodologies (ii) cost-effective technologies for AMR detection (iii) effective alternatives to antibiotic growth promoters and (iv) appropriate biosecurity measures to improve herd health and reduce the use of antimicrobials in farm animals. This project aims to enable developing member states (MSs) use innovative nuclear and related approaches for enhancing the efficiency and effectiveness of national AMR surveillance programmes and promoting good husbandry practices to mitigate AMR in animal production settings. Specifically, it aims to (i) develop, evaluate and validate farm-level sampling methods for detection of AMR in high and low-input animal production environments (ii) establish AMR distribution characteristics in high and low input animal production environments using nuclear, molecular and microbiological techniques (iii) assess the efficacy of alternatives to antibiotic growth promoters (AGPs) as feed additives in animal production settings (iv) establish scientific evidence on development and transmission of AMR at animal-human-environment interface (v) evaluate and optimize phenotyping and genotyping methodologies related to drug resistance in animal infections other than bacteria (vi) pilot and recommend good husbandry practices or antimicrobial stewardship that aim to reduce the risk of emergence and occurrence of AMR in farm animal settings. Three major animal production systems viz. pig, chicken and cattle will be targeted. Nuclear techniques like Raman spectroscopy based stable isotope probing (SIP) and stable isotope linked amino acids (SILAC) will be used to develop novel phenotyping and genotyping methods for AMR characterization. Isotopic methods involving 60Cobalt will be used to produce metabolically active but non-replicative bacteria as candidate para probiotic and potential alternative to antibiotic growth promoters. The project will run for five years and will involve 8 Research Contract (RC) holders from developing countries, three Technical Contract (TC) holders and three Research Agreement (RA) holders from laboratories engaged in high level research on AMR and One Health.

Technical Cooperation Projects

Country TC Number	Description	Technical Officer(s)
Algeria ALG5032	Strengthening the Capacity of the Central Veterinary Laboratory, Regional Laboratories and the Early Warning Laboratories in the Detection, Confirmation of Diagnosis and Surveillance of Animal and Zoonotic Diseases	I. Naletoski
Angola ANG5016	Recovering the Vaccine Production Unit and Monitoring Active Animal Immunity	V. Wijewardana C. Bravo de Rueda
Angola ANG5017	Optimizing Pasture Utilization for Improved Livestock Productivity	V. Tsuma
Burundi BDI5002	Improving Animal Production Through Enhanced Application of Nuclear and Related Techniques	C. Bravo de Rueda I. Naletoski V. Tsuma
Benin BEN5014	Improving Sheep and Pig Productivity and Livestock Traceability	V. Tsuma
Burkina Faso BKF5022	Improving Local Poultry and Local Goat Productivity through Health, Diet, Reproduction, Genetic Markers for Selection and Breeding Management	V. Tsuma
Bosnia and Herzegovina BOH5003	Using Nuclear Technology in Enhancing Science Based Safety, Quality and Control Systems in Feed and Food Chains	L. Porfiri
Central African Republic CAF5010	Building National Capacities for the Diagnosis and Control of Animal Diseases and for Increasing Animal Production	C. Bravo de Rueda
Chad CHD5010	Eradicating Pests in Small Ruminants Using Nuclear Technology	C. Bravo de Rueda
Chad CHD5008	Improving Bovine Productivity Using Artificial Insemination	V. Tsuma
Chile CHI0022	Building Capacity for Nuclear Science and Technology Applications	C. Bravo de Rueda
Cameroon CMR5024	Improving Goat and Sheep Productivity in Rural Areas Using Nuclear-Derived Techniques for Genetic Marker Identification, Reproduction Harnessing and Feed Analysis	V. Tsuma
Dominican Republic DOM0006	Building and Strengthening the National Capacities and Providing General Support in Nuclear Science and Technology	C. Bravo de Rueda
El Salvador ELS5014	Strengthening National Capacities for the Control of Brucellosis	I. Naletoski
Grenada GRN0001	Building National Capacity through the Applications of Nuclear Technology	V. Tsuma
INT5157	Supporting National and Regional Capacity in Integrated Action for Control of Zoonotic Diseases	I. Naletoski
Côte d'Ivoire IVC5043	Applying Nuclear and DNA-Based Techniques to Improve Productivity of Local Livestock	V. Tsuma
Cambodia KAM5009	Improving Livestock Productivity and Control of Transboundary Animal Diseases	V. Tsuma K. Perisamy
Kenya KEN5039	Using Nuclear and Nuclear Related Technologies for Sustainable Livestock Productivity	V. Tsuma

Country TC Number	Description	Technical Officer(s)
Kyrgyzstan KIG5001	Establishing Effective Testing and Systematic Monitoring of Residues and Food Contaminants and of Transboundary Animal Diseases	I. Naletoski
Lao P.D.R. LAO5007	Strengthening National Animal Health Laboratory Network	C. Bravo de Rueda
Madagascar MAG5027	Improving Livestock Production through Artificial Insemination and Disease Control	V. Tsuma
North Macedonia MAK5011	Improving National Capacities for Early Detection and Characterization of Emerging and Re-emerging Animal Diseases with Strong Economic Consequences and Upgrade of the Bio Risk Management at the National Laboratory	I. Naletoski
Malaysia MAL5034	Strengthening National Capacity and Capability in Nuclear and Molecular Techniques in Supporting Transboundary Animal and Zoonotic Diseases of Veterinary Public Health Significance	C. Bravo de Rueda
Mexico MEX5033	Sustainable Production of Sheep and Goats in Mexico using Nuclear and Nuclear Related Techniques	V. Tsuma
Malawi MLW5004	Strengthening Capacity for the Diagnosis and Control of Mastitis in Dairy Cattle	C. Bravo de Rueda
Montenegro MNE5005	Enhancing Capacity of the National Veterinary Laboratory for Detection of Highly Contagious Animal Diseases	I. Naletoski
Mongolia MON5026	Improving the Diagnosis and Treatment of Transboundary Animal Diseases with Potential Pandemic Patterns	C. Bravo de Rueda
Mozambique MOZ5011	Using Nuclear and Nuclear Related Techniques to Improve Animal Health and Breeding	C. Lamien
Myanmar MYA5030	Advancing National Capacities to Detect and Respond to Transboundary Animal Diseases	C. Bravo de Rueda T.B. Settypalli
Nepal NEP5008	Reducing the Incidence of Brucellosis in Animals and Humans through Surveillance and Control	I. Naletoski
Vanuatu NHE5003	Enhancing Livestock Production and Health	V. Tsuma C. Bravo de Rueda
Nigeria NIR5041	Improving Livestock Productivity through Enhanced Nutrition and Reproduction Using Nuclear and Molecular Techniques	V. Tsuma
Oman OMA6009	Building and Strengthening Technical Capacity to Prevent and Respond to Outbreaks of Viral Diseases	C. Bravo de Rueda I. Naletoski
Pakistan PAK5052	Improving Livestock Productivity Using Nuclear and Related Techniques by Exploiting Indigenous Feed Resources while Reducing Enteric Greenhouse Gas Emissions	C. Bravo de Rueda
Pakistan PAK5053	Strengthening and Enhancing National Capabilities for the Development of Climate Smart Crops, Improvement in Animal Productivity and Management of Soil, Water, and Nutrient Resources Using Nuclear and Related Techniques	V. Tsuma C. Bravo de Rueda
Papua New Guinea PAP5004	Improving Reporting of the Incidence and Prevalence of Animal Health and Diseases Using Nuclear Derived Techniques	I. Naletoski
Paraguay PAR5011	Improving the Conservation of Germplasm of High-Performance Livestock and Native Cattle	V. Tsuma
Peru PER5035	Improving Pasture Production Through Best Soil Nutrient Management to Promote Sustainable Livestock Production in the Highland Region	V. Tsuma

Country TC Number	Description	Technical Officer(s)
Palau PLW5004	Establishing Technical Capability in Animal Production and Disease Control	C. Bravo de Rueda
Congo PRC6002	Contributing to the Epidemiological Surveillance of Neglected Tropical Diseases	C. Bravo de Rueda
RAF5082	Enhancing Veterinary Diagnostic Laboratory Biosafety and Biosecurity Capacities to Address Threats from Zoonotic and Transboundary Animal Diseases (AFRA)	I. Naletoski
RAF5089	Strengthening the Capacities of National Veterinary Laboratories for the Early Warning, Control and Prevention of Outbreaks of Animal and Zoonotic Diseases (AFRA)	C. Bravo de Rueda
RAF5090	Supporting Climate Change Adaptation for Communities Through Integrated Soil–Cropping–Livestock Production Systems (AFRA)	V. Tsuma
RAS5085	Using Nuclear Derived Techniques in the Early and Rapid Detection of Priority Animal and Zoonotic Diseases with Focus on Avian Influenza	I. Naletoski
RER5027	Enhancing Preparedness Capacities of the Veterinary Sector to Confront with Emerging and Re-emerging Diseases of Livestock and Wildlife	I. Naletoski
RLA5084	Developing Human Resources and Building Capacity of Member States in the Application of Nuclear Technology to Agriculture	C. Bravo de Rueda
RLA5085	Strengthening the Capacity of Official Laboratories for Monitoring and Response to an Outbreak of Priority Animal and Zoonotic Diseases (ARCAL CLXXIV)	C. Bravo de Rueda I. Naletoski
RLA5086	Decreasing the Mortality Rate of Rainbow Trout Associated with Infectious Pancreatic Necrosis Virus and Emerging Diseases Using Molecular and OMIC Techniques (ARCAL CLXXV)	C. Bravo de Rueda
Senegal SEN5042	Using Nuclear and Related Techniques in Improving the Productivity of Domestic Ruminants	V. Tsuma
Sierra Leone SIL5022	Enhancing Livestock Production and Artificial Insemination Programme to Increase Milk and Meat Production in Cattle	V. Tsuma
Sri Lanka SRL5049	Supporting Control of Stomach Worm Infection in Goats	C. Bravo de Rueda V. Wijewardana
Kingdom of Eswatini SWA5001	Reducing the Incidence and Impact of Transboundary Animal and Zoonotic Diseases	C. Bravo de Rueda
Syrian Arab Republic SYR5025	Enhancing the Nutritive and Reproductive Characteristics of Small Ruminants by Means of Nuclear and other Related Techniques Using Locally Available Unconventional Feed Resources	V. Tsuma
Tajikistan TAD5006	Applying Nuclear and Molecular Techniques for Diagnosis and Control of Transboundary Animal Diseases	I. Naletoski
Togo TOG5005	Enhancing Animal Production Using Artificial Insemination	V. Tsuma
Tunisia TUN5032	Establishing a National Certified Pipeline to Produce Aquaculture Vaccines by Irradiation	V. Wijewardana R. Kangethe
U.R. of Tanzania URT5036	Enhancing Artificial Insemination Services and Application of Radioimmunoassay Techniques to Improve Dairy Cattle Productivity	V. Tsuma
Uruguay URU5030	Introducing Genetic Traceability Technology for Improved Food Safety	V. Tsuma

Country TC Number	Description	Technical Officer(s)
Viet Nam VIE5024	Strengthening Diagnosis, Surveillance, and Control of Emerging Transboundary Animal and Zoonotic Diseases with Emphasis on African Swine Fever and Severe Acute Respiratory Syndrome Coronavirus 2	C. Bravo de Rueda
Viet Nam VIE5025	Applying Nuclear Related Technology for Selecting Climate Adapted Indigenous Swine and Chicken Breeds	V. Tsuma
Zimbabwe ZIM5024	Establishing an Artificial Insemination Center to Enhance the Rebuilding of the National Herd	V. Tsuma
Zimbabwe ZIM5025	Producing Theileriaparva and Other Tick Borne Disease Vaccines	C. Bravo de Rueda

Publications

Publications in Scientific Journals

Ankhanbaatar U, **Auer A**, Ulziibat G, et al. Comparison of the Whole-Genome Sequence of the African Swine Fever Virus from a Mongolian Wild Boar with Genotype II Viruses from Asia and Europe. Pathogens. 2023;12(9):1143. DOI: 10.3390/pathogens12091143. PMID: 37764951; PMCID: PMC10536492

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Tully M, Batten C, Ashby M, Mahapatra M, Parekh K, Parida S, Njeumi F, Willet B, Bataille A, Libeau G, Kwiatek O, Caron A, **Berguido FJ**, **Lamien CE**, Cattoli G, Misinzo G, Keyyu Ju, Mdelete D, Gakuya F, Bodjo C, Haha FA, Elbashier HM, Khalafalla AI, Osman AY, Kock T. The evaluation of five serological assays in determining seroconversion to peste des petits ruminants virus in typical and atypical hosts. Sci Rep 13, 14787 (2023). https://doi.org/10.1038/s41598-023-41630-3

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