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Nuclear Techniques in Food and Agriculture

Animal Production & Health Newsletter



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To Our Readers



The Animal Production and Health Team honoured by the IAEA “One-House” Superior Achievement award 2021 for its COVID-19 support actions: (from left to right) Mr Ivancho Naletoski, IAEA-DG Rafael Mariano Grossi, Mr Gerrit Viljoen, Mr Charles Lamien and Mr Giovanni Cattoli

Dear colleagues,

For more than 50 years, the FAO and the IAEA have been expanding knowledge and enhancing capacity in leveraging nuclear sciences to help feed the world and recently strengthened their partnership, creating a Joint FAO/IAEA Centre of Nuclear Techniques in Food and Agriculture.

The Joint FAO/IAEA Centre together with IAEA colleagues from three departments (Nuclear Applications and Techniques, Technical Cooperation, and Management) have been recognized for assisting Member States in the early detection of SARS-CoV2 and the control of COVID-19. Under the motto “One-House for One-Health”, the IAEA ‘One-House’ award 2021 underlines how effective teamwork can support the timely detection, diagnoses, and control of animal and zoonotic disease outbreaks.

Building on the longstanding experience with transboundary animal and zoonotic disease outbreaks, the Animal Production and Health Team of the Joint FAO/IAEA Centre, through its Animal Production and Health (APH) Laboratory and the FAO/IAEA Veterinary Diagnostic Laboratory (VETLAB) Network, has been closely cooperating with veterinary laboratories in the field to assist them with the COVID-19 outbreak.

This was the largest technical support initiative since the agency's foundation amounting over 36 million euros to fight the pandemic. Among the achievements were:

- The Agency provided COVID-19 emergency packages to 129 IAEA Member States with full technical advice and backup from the Joint FAO/IAEA Centre.
- The Agency provided guidance and expert services to 305 medical and veterinary laboratories involved on COVID-19 testing.
- The Joint FAO/IAEA Centre, through its APH Laboratory and the VETLAB Network, provided timely guidance and validated procedures on COVID-19 detection to 124 veterinary laboratories in 46 Member States.
- The Joint FAO/IAEA Centre provided direct one-on-one technical back stopping to 87 veterinary laboratories.

COVID-19 remains a great threat to humanity as the availability of vaccines in many countries is still below what is needed and misinformation about the vaccines fuels scepticism in part of the population around the world.

Since the duty travels are still highly restricted, the staff of the Joint FAO/IAEA Centre is conducting frequent virtual meetings with our project counterparts and other entities. This allows us to stay informed about the activities in our Technical Cooperation Projects (TC), Coordination Research Projects (CRPs) and scientific world, to provide technical advice, guidance, support and training as well as the procurement of equipment and building capacities.

Fortunately, capacity building in the form of fellowship trainings and training courses with our experts has been possible. The former took place mainly within the African region, and the latter in South America. One of the highlights was a training course for the *Early and Rapid Detection of African Swine Fever in Pigs* conducted in the Dominican Republic in October 2021 due to the recent outbreak in the country.

The training was physically attended by 24 local trainees and two experts from Spain. The online version of the training brought together more than 200 participants and experts from the Latin American region and Spain dealing with prevention, control and diagnostics of African swine fever (ASF). Important to note - it is the first case of ASF in the region.

Outbreaks of transboundary animal and zoonotic diseases continued affecting countries around the globe. We provided trainings in response to emergency requests by Member States (MSs) on laboratory technologies for the early and rapid diagnosis, including pathogen sequencing for the highly pathogenic avian influenza H5N6, Rift Valley fever in Africa, lumpy skin disease and ASF in several regions including Europe, as well as for peste des petits ruminants (PPR) and foot-and-mouth disease, to mention a few.

As always, the Animal Production and Health Laboratory in Seibersdorf has been crucial in supplementing expertise and research guidance. However, training courses were not just focussed on transboundary animal diseases, as national training courses were also carried on in Ethiopia on feed supplementation and in Burundi on assisted reproductive technologies.

We have been actively finalizing work plans and budgets for the next cycle of TC projects starting in 2022, based on the approval given during the 65th IAEA General Conference in September 2021 in Vienna. To date we have nearly 60 new TC projects concerning animal nutrition, reproduction, breeding and health on various livestock species including poultry and fish. So far, we have made good progress in coordinating and evaluating proposals for research agreements and research contracts for our four new CRPs approved for 2022:

- Application of Advanced Molecular Characterization Technologies Through the Veterinary Diagnostic Laboratory Network (VETLAB Network)
- Novel Test Approaches to Determine Efficacy and Potency of Irradiated and Other Vaccines
- Improving Efficiency of Animal Breeding Programs Using Nuclear Related Genomic Information
- Nuclear and Related Techniques to Measure the Impact of Type of Feeding and Production System on Greenhouse Gas (GHG) Emissions and Livestock Productivity

These four new CRPs will provide plenty of research excitement and valuable results for improving livestock productivity, health control and food security in developing countries.

The **Zoonotic Disease Integrated Action (ZODIAC)**, the largest technical project of the IAEA since its inception, was launched in June 2020 and is now in full swing. Nearly 150 MS of the IAEA have joined the initiative, which aims to provide the world and specifically diagnostic laboratories with tools to identify and characterize zoonotic disease pathogens early, before they can spread and develop into an outbreak with pandemic potential such as COVID-19. Several meetings were held to explore the priority disease pathogens and for developing tools for their mining, monitoring and tracing.

In the first half of the year, we conducted one meeting with African scientists. In the second half, we held meetings with scientists from the Americas and the Caribbean region from Asia and Pacific and from Europe and Central Asia. In the near future, to target regional needs, the CRPs will be developed with participation from experts and counterparts from each region.

We are also working on a manual focusing on the most effective sample materials, sampling techniques, sample preparation for the various subsequent pathogen detection methods, including biosafety issues, and best and safe shipment options.

In addition, the Animal Production and Health Subprogramme continues its focus on enhancing food security by supporting sustainable livestock production systems in developing countries. This is to be achieved by strategic and applied research, technology transfer and capacity building. The three principal components of the subprogramme are animal nutrition, animal reproduction, breeding, and genetics; and animal health. Animal production and health problems are identified, and solutions developed by strategically applied isotopic, nuclear, nuclear-based and nuclear-derived tools, in conjunction with conventional technologies to:

- Characterize and optimally utilize the nutritional value of locally available feed and feed resources to enhance energy conversion, whilst protecting the environment and minimizing greenhouse gas emissions;

- Enhance animal reproduction and breeding through the introduction of artificial insemination, embryo transfer and productive breed selection, and the characterization of livestock genetic make-up to drive the integration of locally adapted animal breeds with trait selected exotic breeds to satisfy the increasing demand for more and better-quality animals and animal products;
- Assess and reduce the risk of transboundary animal and zoonotic diseases to livestock and livestock owners through the implementation of early and rapid diagnosis and control technologies and their use in national and international control and eradication programmes.

The above activities are complemented by tools developed for computerized data management in disease diagnosis and animal production; use of geographic information systems in management of farm resources and diseases; and distance learning through information communication technologies in the related areas. The FAO/IAEA Veterinary Diagnostic Laboratory (VETLAB) Network is instrumental for the development, validation and dissemination of technologies, know-how and expertise worldwide.

We hope that some of our activities will be back to a new “COVID-19 normal” during the first half of 2022, allowing for in-person meetings and country visits. In the meantime, please be cautious and keep healthy measures.

All the best for 2022.



Gerrit Viljoen

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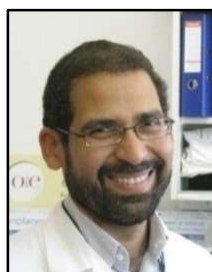
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VETLAB

Network Bulletin



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**VETLAB is an initiative of the
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To the readers

Despite all the restrictions and limitations imposed by the current pandemic situation, the VETLAB Network proved to be productive and filled with several activities. In fact, the active contributions of network's partners resulted in ten scientific papers sharing relevant data and information on the porcine circovirus (PCV) emergence and circulation in Africa. The published papers also shared novel data on the molecular epidemiology of African swine fever (ASF) virus and peste des petits ruminants (PPR) virus in Africa and Asia. Scientific research was conducted on pox viruses and a novel luciferase immunoprecipitation system (LIPS) platform was applied and tested for the SARS-CoV2 antibody detection in various animal species. Because this assay is species-independent and requires a tiny amount of serum it can be very useful for the sero-surveillance in animals, particularly wildlife species, as well as for serology in small laboratory animals. On top of that, the VETLAB Network provided emergency support and technical assistance to the African countries most recently affected by H5N1 avian influenza virus.

This year, in satisfaction of everyone involved, the network was able to organize the annual meeting of the VETLAB Network laboratories directors'. Although the COVID-19 situation imposed the organization of this meeting online, it represents a positive step forward (last year it was not possible at all to organize it) and we all hope to have our meeting on-site again, in 2022. You can find more details and the latest information in the current issue of the bulletin and in the APH Newsletter.

Now it's time for the APH Team to wish you all, your families and colleagues, a happy and healthy 2022. We look forward for your brilliant network collaboration and contributions in the new coming year.

VETLAB Highlights

Molecular epidemiology of Porcine Circovirus (PCV) and Co-infection of PCV and African swine fever virus (ASFV)

The VETLAB Network analysed ASFV and porcine circovirus 2 (PCV-2) co-infections in 10 African countries. Four genotypes were identified (i.e., PCV-2a, PCV-2b, PCV-2d, PCV-2g) showing the existence of several African-specific clusters and determining the estimated time of introduction of PCV-2s into Africa (published in *Transbound Emerg Dis* [6]). Another study in Indonesia and Mongolia enabled the detection of ASFV and PCV-2 co-infections in pigs (published in *Arch Virol* [5]). A separate study in Mozambique led to the discovery of ASFV and PCV-3 co-infection (published in *Vet Res Commun* [1]).

Molecular epidemiology of PPR in Bangladesh

The Department of Pathology of Bangladesh Agricultural University, in collaboration with APHL, undertook the molecular evolutionary analysis of PPRVs circulating in Bangladesh between 2008-2020. The results showed that all PPRV in the country belong to lineage IV and are closely related to Chinese and Indian isolates. This study also reported the prediction of 16 epitopes on 4 immunogenic proteins of Bangladeshi PPRV (published in *Infect Genet Evol* [9]).

Avian Influenza H5N1 in Southern Africa (Botswana and Lesotho)

After the occurrence of highly pathogenic avian influenza outbreaks (H5N1) in Southern Africa in July/August 2021, the VETLAB Network provided technical assistance to Lesotho and Botswana for the genetic characterization of the virus. The genome sequences of the first-ever reported H5N1 in the two countries were sequenced and analyzed.

Novel serological platform for detection of SARS-CoV2 antibodies in animal species

APHL, in collaboration with the National Institutes of Health, Bethesda, Maryland, USA, the Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro, Italy, and the Friedrich-Loeffler-Institut, Greifswald-Insel Riems, Germany, has evaluated the suitability of the luciferase immunoprecipitation system (LIPS) technology for the detection of SARS-CoV-2 for antibodies in various animal species. The SARS-CoV-2-LIPS-S assay based on the spike protein provided better discrimination between the positive and negative samples than the SARS-CoV-2-LIPS-N assay based on the nucleoprotein. This study showed the suitability of the SARS-CoV-2-LIPS-S assay for the sero-surveillance of SARS-CoV-2 infection in a range of animal species (published in *Viruses* [3]).



FAO Training course on LSD diagnosis - Indonesia

The VETLAB partners in Indonesia (BBalitVet, Bogor) and one expert from APHL, Seibersdorf provided assistance for this training course held in Subang-Java on 19-21 October 2021

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VETLAB Capacity Building Initiatives

with the objective of strengthening laboratory preparedness for the early detection of the disease in this at-risk country. APHL also provided the laboratory procedures and the positive controls to be distributed to 11 national laboratories.

Training Course for Veterinary Diagnostic Laboratory Network Partners on Sequencing and Bioinformatics

The training aimed to strengthen the capacity of the VETLAB Network partner laboratories in using conventional and new sequencing technologies and bioinformatic tools for the accurate identification of pathogens. It was held online on 29 November to 10 December 2021. 18 participants from 9 partner laboratories attended the course which consisted of theoretical lessons and practical exercise on the analysis of next generation sequencing data on the Linux interface and phylogenetic analysis.

VETLAB Networking Activities

Interlaboratory test for the diagnosis of PPR

The 2021 interlaboratory comparison exercise to assess countries' diagnostic capacity for the accurate detection of PPR is in progress. Twenty-nine laboratories from 27 countries accepted the invitation to participate and shipment of the panels is in due course. Due to the current epidemiological situation in the East Asian region, this year a panel for Lumpy skin disease virus (LSDV) molecular detection is added for some Asian laboratories.

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

The purpose of the meeting was to update partners on the activities of the VETLAB Network and to discuss the main challenges and gaps in implementing animal and zoonotic disease diagnosis. The online meeting took place from 11 to 15 October 2021.

VETLAB Network Laboratories:

Central Veterinary Laboratory (CVL), Windhoek-Namibia

The Central Veterinary Laboratory (CVL) has been the national veterinary laboratory operator for more than 30 years. Its scope of activities are diagnostic services, biotechnology/research, and food science. The service portfolio includes disease diagnostic services (i.e. post mortem and histopathological examinations, serological analysis, clinical microbiological analysis, and molecular diagnostic methods). CVL also provides analytical and diagnostic services to the agricultural industry to ensure export/import certifications of livestock and livestock products and are thus a key player in safe trade of Namibia's meat products.

The CVL is a major contributor to the One Health approach by strengthening the country's zoonotic disease testing and surveillance activities, especially for rabies, anthrax, brucellosis, and bovine tuberculosis, among many others. During the unprecedented COVID-19 pandemic, the CVL has been providing COVID-19 testing, supplementing the national response. In the past five years, the laboratory has renewed their accreditation to ISO IEC 17025 Standard while enhancing their service delivery by upgrading the laboratory information system known as SILABFA.

More recent VETLAB publications

1. Anahory, I.V., et al. Identification of porcine circovirus-3 in Mozambique. *Vet Res Commun.* Nov 8 (2021). DOI: 10.1007/s11259-021-09858-4
2. Ankanbaatar, U., et al. Isolation and Identification of a Highly Pathogenic Avian Influenza H5N6 Virus from Migratory Waterfowl in Western Mongolia. *J Wildl Dis.* Oct 26 (2021). DOI: 10.7589/JWD-D-21-00032

3. Berguido, F.J., et al. Serological detection of SARS-CoV-2 antibodies in naturally-infected mink and other experimentally-infected animals. *Viruses* 13:1649 (2021). DOI: 10.3390/v13081649

4. Chibssa, T.R., et al. Innate Immune responses to wildtype and attenuated sheppox virus mediated through RIG-1 sensing in PBMC in-vitro. *Frontiers in Immunology* 12 (2021). DOI: 10.3389/fimmu.2021.666543

5. Dundon, W.G., et al. Evidence of coinfection of pigs with African swine fever virus and porcine circovirus 2. *Arch Virol* (2021). DOI: 10.1007/s00705-021-05312-7

6. Franzo, G., et al. Porcine circovirus-2 in Africa: Identification of continent-specific clusters and evidence of independent viral introductions from Europe, North America and Asia. *Transbound Emerg Dis* (2021). DOI: 10.1111/tbed.14400

7. Minoungou, G.L., et al. Molecular characterization of African Swine fever viruses in Burkina Faso, Mali, and Senegal 1989–2016. Genetic diversity of ASFV in West Africa. *Transbound Emerg Dis* 68:2842–2852 (2021). DOI: 10.1111/tbed.14240

8. Modise, B.M., et al. First molecular characterization of poxviruses in cattle, sheep, and goats in Botswana. *Virology J* 18: 167 (2021). DOI:10.1186/s12985-021-01634-9

9. Nooruzzaman, M., et al. Molecular insights into peste des petits ruminants virus identified in Bangladesh between 2008 and 2020. *Infect Genet Evol.* Nov 27 (2021). DOI: 10.1016/j.meegid.2021.105163.

10. Paweska, J.T., et al. Large-Scale International Validation of an indirect ELISA based on recombinant nucleocapsid protein of Rift Valley fever virus for the detection of IgG antibody in domestic ruminants. *Viruses* 13:1651 (2021). DOI: 10.3390/v13081651



The Central Veterinary Laboratory in Windhoek, Namibia

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Forthcoming Events

Group Fellowship on New Generation Sequencing Using the Ion Torrent S5 Platform (INT5157 – Pillar 1 of the ZODIAC Initiative)

Ivancho Naletoski

A series of group fellowships on the use of the selected New Generation Sequencing (NGS) platforms in the local ZODIAC National Laboratories (ZNLs) will be organized by the IAEA as a follow up activity to the delivery of the NGS Platforms under Pillar 1 of the ZODIAC Initiative.

The first group fellowship will be dedicated to the NGS Ion Torrent S5 platform. The fellowships will be hosted by the IAEA APH laboratory in Seibersdorf, Austria. The fellows will receive practical training in sample preparation, extraction of nucleic acids and quality control of the extracts, library preparation, template preparation, sample runs, downloading and quality control of raw data received from the readings and bioinformatic processing of the results.

The fellowship event was opened in November 2021. Eligible candidates from the ZNLs in Tunisia, Senegal and Indonesia are invited to submit applications. The implementation is expected to start during the first quarter of 2022.

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

Victor Tsuma and Mario Garcia Podesta

This is a first meeting of the new Coordinated Research Project (CRP) on applying nuclear and related genomic technologies for enhancing the efficiency of national dairy cattle breeding programmes, taking place at the IAEA Headquarters in Vienna, Austria from 4 to 8 April 2022.

The aim of the meeting is to discuss objectives and major activities, share national data on cattle breeding programmes, and to discuss and update workplans of individual research contracts.

Research contract holders will present their national dairy animal breeding and development programs, including infrastructure and technologies related to artificial insemination (AI) services.

Agreement holders will present recent advances in animal breeding methodologies, while outlining opportunities and challenges for application. The Joint FAO/IAEA Centre for Nuclear Technologies in Food and Agriculture will present the possible usage of existing tools and resources that have been developed or optimized at the Animal Production and Health Laboratories (APHL), Seibersdorf, Austria.

Read more about the scope of this CRP on page 34 of this edition.

First Research Coordination Meeting on Nuclear and Related Techniques to Measure the Impact of Type of Feeding and Production System on Greenhouse Gas (GHG) Emissions and Livestock Productivity (D31031)

Victor Tsuma and Mario Garcia Podesta

This is a first meeting of the new Coordinated Research Project (CRP) on applying nuclear and related technologies and resources to optimize livestock feeding practices that reduce greenhouse gases (GHG) emissions and help mitigate climate change, taking place at the IAEA Headquarters in Vienna, Austria from 25 to 29 April 2022.

The aim of the meeting is to discuss objectives and major activities, share data on national cattle production systems and methane conversion factors of the Intergovernmental Panel on Climate Change (IPCC), and to discuss and update workplans of individual research contracts.

Research contract holders will present their national cattle production programmes including facilities and skills. An overview of the feeding practices in relation to their environmental impact and sustainable productivity will be given. Agreement holders will present data on recent advances in livestock feeding and nutrition strategies to reduce GHG emissions, and practical methods for assessing livestock GHG emissions.

Read more about the scope of this CRP on page 35 of this edition.

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

Gerrit Viljoen and Viskam Wijewardana

This is a first meeting of the new Coordinated Research Project (CRP) on novel approaches to determine efficacy of vaccines and immune mechanisms underlying novel vaccines, taking place at the IAEA Headquarters in Vienna, Austria, from 16 to 20 May 2022. The purpose of the event is to discuss proposed work plans of the participants in this new CRP, which focuses on methods to evaluate irradiated and other novel vaccines by using innovative tools to determine their immune response.

It is expected that the participants will bring new and original ideas and approaches on designing immunological tools for quality control and efficacy assessment of experimental vaccines. Eight to ten contract holders and interested observers will participate in the first meeting.

Read more about the scope of this CRP on page 37 of this edition.

Past Events

Virtual Regional Training Course on Genetic characterization of livestock breeds - Bioinformatics analysis of multi locus genotype data (BKF5021)

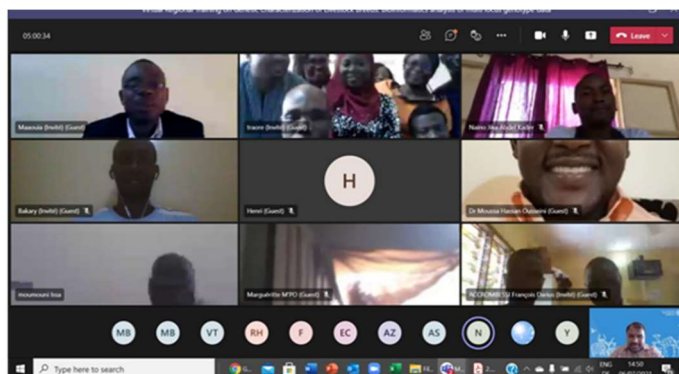
Kathiravan Periasamy

As part of Burkina Faso's National Action Plan on Animal Genetic Resources and the Technical Cooperation project Improving Local Poultry Production Through Incorporation of Nutraceuticals in Feeds and Genetic Characterization (BKF5021), a regional virtual training course was organized from 6 to 10 July 2021. The training course was attended by twenty-six participants from five countries in West Africa (7: Burkina Faso, 6: Niger, 6: Côte d'Ivoire, 4: Senegal, 3: Benin).



Virtual regional training course on bioinformatics data analysis for West African countries

The course included lectures and practical hands-on training on molecular genetic characterization, covering among others the following aspects: i) extraction of multi locus genotype data for genetic characterization of livestock breeds, ii) estimation of basic biodiversity indices of local breeds, iii) estimation of genetic distance and construction of phylogeny, iv) evaluation of population structure and estimation of levels of genetic admixture, v) introduction to genome-wide technologies for genetic characterization of livestock breeds. The training was expected to help improve the regional capacity in applying molecular techniques for genetic characterization of indigenous livestock breeds in West Africa.



Virtual regional training course on bioinformatics data analysis for West African countries

Virtual National Training Course on Phenotype recording and conventional breeding methods for Cashmere goat improvement (MON5025)

Kathiravan Periasamy

As part of Mongolia's national Technical Cooperation project Improving Breed Characterization of Cashmere Goats to Facilitate the Establishment of Strategic Breeding Programs (MON5025), a virtual training course was organized from 14 to 25 June 2021. The training course was attended by eleven participants from various institutions in Mongolia.

The course included lectures and practical hands-on training on: a) animal identification, phenotype recording and digitization of data in institutional farms and farmers' flocks, b) strategies for selection and breeding of goats to improve cashmere production, c) introduction to basic concepts of quantitative genetics in animal breeding, d) estimation of genetic parameters and conventional breeding values, e) introduction to genomics and potential applications for genetic improvement of livestock, and f) applying genomics to estimate genetic distance, phylogeny, population structure and genetic admixture.

Prof. Johann Sölkner and Dr. Gabor Meszaros (Division of Livestock Sciences, University of Natural Resources and Life Sciences, Vienna, Austria) served as expert lecturers for the training course. The training course was expected to help improve the national capacity on performance data recording and breeding for cashmere wool production in Mongolia.



Virtual national training course on breeding for cashmere wool production in Mongolia

National Training Course on Artificial Insemination in Cattle and Reproductive Status Evaluation including Early Pregnancy Diagnosis, Burundi

Victor Tsuma

The national training course on Artificial Insemination in Cattle and Reproductive Status Evaluation including Early Pregnancy Diagnosis was held in Bujumbura, Burundi from 16 to 27 August 2021. The aim of the course was to provide hands-on practice of bovine artificial insemination (AI), and ultrasonographic evaluation of the female reproductive tract.

The 10-day training course was attended by thirty animal health practitioners from various regions of the country. Prof. Moumouni Issa, from Université Abdou Moumouni, Niamey, Niger, and Dr. Wilkister Nakami Nabulindo from the University of Nairobi, Kenya, were the expert trainers leading the course. The training consisted of presentations, question and answer sessions, demonstrations using abattoir-sourced reproductive organs, and hands-on practice on live animals.

The course provided an overview of the bovine anatomy and reproductive physiology as it relates to AI and ultrasonography; 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; trouble-shooting causes of AI failure; and, bovine female reproductive tract ultrasonography including pregnancy diagnosis.



The trainees with the facilitators (front row standing 4th and 5th from left) during the launch of the training programme by the Director General, Ministry of Livestock, Burundi

Consultancy Meeting on Development of Tools for the Mining, Monitoring and Tracing of Zoonotic Pathogens in the Americas & Caribbean, Europe & Central Asia and Asia & Pacific Areas

Charles Lamien

Three virtual consultation meetings on Development of Tools for the Mining, Monitoring and Tracing of Zoonotic Pathogens in the Americas & Caribbean, Europe & Central Asia and Asia & Pacific Areas were held at the end of Summer 2021: a) 30 August 2021 for the Americas and Caribbean, b) 1 September 2021 for Asia and Pacific, and c) 3 September 2021 for Europe and Central Asia.

The purpose of these meetings was to discuss and identify priority zoonotic disease pathogens and the laboratory tools and technologies needed for their mining, monitoring, tracing, and characterization to perform comprehensive field studies through multiple competent laboratories in the framework of the Supporting National and Regional Capacity in Integrated Action for Control of Zoonotic Diseases (ZODIAC) project.

The virtual meetings provided a forum of exchange for the external experts and key international organizations involved in zoonoses research and control, including the surveillance in diseases reservoirs and vectors and livestock in the respective regions.

The participants provided a review and identified the optimal approaches and tools for sample collection, storage, disease surveillance, and pathogen mining that had the most significant potential for adaptation through associated Coordination Research Projects (CRPs) that have been developed for each of the regions.

The tools and approaches will be transferred to the ZODIAC national laboratories to improve disease surveillance and monitoring in the countries and regions.

The experts also identified critical R&D gaps which could be addressed by the CRPs, along with defining the priority diseases, pathogens and the essential species to be included in surveillance. Last but not least, the experts also formulated certain recommendations to improve the research agenda of the CRPs. The document reporting the outcomes of this meeting was reviewed by the experts and will serve as the basis for the formulation of the CRPs for each of the targeted regions.

National Training Course on Livestock Forage Production and Conservation Techniques (ERI5010)

Victor Tsuma

The national training course on Livestock Forage Production and Conservation Techniques was held in Asmara, Eritrea from 18 to 29 October 2021. The aim of the course was to offer an overview of sustainable livestock production and nutrition systems in relation to available forage resources, participatory livestock forage resource mapping and land use planning, forage production systems/practices for sustainable livestock production, and forage/feed conservation technologies in pastoral and agropastoral production systems.



Field learning on hay as an option for fodder conservation

The 10-day training course was attended by twenty-two participants from across the country dealing with agricultural extension, animal production and nutrition, livestock breeding, and rangeland management in various capacities, including policy level.

Among the trainees were the staff from the Agricultural Extension Department (AED) and the Natural Resources Department of the Eritrean Ministry of Agriculture, and the National Agricultural Research Institute (NARI) of Eritrea. Dr. Oscar Kipchirchir Koech from the Department of Land Resource Management and Agricultural Technology (LARMAT), University of Nairobi, Kenya, was the expert trainer of the course.

The participatory learning approach was the basis of various modes of delivery, including lectures, demonstrations, practical and hands-on training on livestock feed resource mapping and feed conservation practices.

Training Course on the Laboratory Diagnosis of African Swine Fever (ASF) in Dominican Republic with Participation of the Regional Laboratories as Observers (DOM0006)

Ivancho Naletoski

In the summer 2021, the outbreak of African swine fever (ASF) in Dominican Republic was confirmed by the Foreign Animal Disease Diagnostic Laboratory, Plum Island, USA, by molecular techniques. The Joint FAO/IAEA Centre provided emergency support to Dominican Republic as requested by the national authorities, in the form of diagnostic support packages and a training course.

The former comprised diagnostic kits for detection of specific antibodies against ASF, diagnostic kits for detection and characterization of the ASF virus, as well as other necessary laboratory equipment and consumables, sampling accessories and personal protective equipment. The latter was an emergency training course for the national laboratories on techniques for early and rapid detection and characterization of the ASF virus, and was held in San Salvador from 25 to 29 October 2021.

Latin America had not experienced ASF outbreaks in decades. Therefore, officially designated veterinary laboratories from the region were invited to follow the training course as observers (virtual, chat). The aim was to familiarize the laboratory operators across the region with the diagnostic procedures for ASF.



Group photo from the national training course in San Salvador, Dominican Republic

The training course was attended in person by thirteen participants from the country, and virtually by 214 participants from 19 Latin American member states (Argentina, Bolivia, Brazil, Chile, Colombia, Costa Rica, Cuba, Ecuador, El Salvador, Guatemala, Guyana, Honduras, Mexico, Nicaragua, Panama, Peru, St. Vincent and The Grenadines, Uruguay, and Venezuela).

Staff members of the Veterinary Surveillance Center (VISAVET) of the Faculty of Veterinary Medicine, Complutense University of Madrid, Spain (UCM), an OIE reference laboratory for ASF, headed by Prof Dr José Manuel Sánchez-Vizcaíno, were the expert trainers of the course.

The hybrid format of the course allowed two experts from VISAVET to hold practical exercises at the local laboratory LAVECEN in Santo Domingo, while the theoretical part of the course was held online.

The lectures and the practical exercises covered the following topics: general aspects and global situation of ASF; main lesions and other clinical signs; laboratory diagnosis; integration of diagnostic techniques in the control of ASF; molecular (conventional and real-time PCR) and serological techniques (ELISA and pen-side tests for antibody detection); and differential diagnostics and the use of Sanger sequencing in the characterization of the circulating ASF strains.

Peste des Petits Ruminants Global Eradication Programme Expert Group Workshop

Charles Lamien and Giovanni Cattoli

In the context of the Peste des Petits Ruminants (PPR) Global Eradication Program (GEP), the APH Laboratory, in collaboration with the Royal Veterinary College (UK) and the participation of the OIE/FAO PPR GEP Secretariat, organized a virtual PPR Expert Group Workshop from 27 September to 1 October 2021. The aims of the workshop were as follows: a) to review the data and current state of knowledge on PPR that has been or could be derived from serological surveillance, and b) to advise participants on what constitutes effective serological assays across host species, as well as on appropriate testing protocols or algorithms for populations during disease surveillance or monitoring for various purposes (e.g. general surveillance, post vaccination monitoring of viral circulation, specific host surveillance etc.).

The workshop offered an opportunity to discuss the state of the art of PPR serology in wildlife and atypical hosts (e.g. camels and pigs). Moreover, the participants were introduced to the application of novel blocking ELISA (bELISA) and serological platforms for PPR, such as LIPS and pseudo-type virus neutralization assay (PVNA) followed by a presentation and comparison of the results obtained from these assays.

Twenty-five experts from international organizations (AU-PANVAC, FAO, IAEA, and OIE), veterinary research institutions and PPR reference laboratories participated in the workshop.

Group Fellowship under the National Project in Tajikistan (TAD5006)

Ivancho Naletoski

Five young scientists from the Institute of Veterinary Medicine (IVM) of the Tajik Academy of Agricultural Sciences, Dushanbe, were granted a group fellowship at the Faculty of Veterinary Medicine, Skopje, North Macedonia, under supervision of Dr Kiril Krstevski and Dr Igor Dzadzovski, between 1 October and 30 November 2021.

The fellowships were awarded to IVM as part of the national Technical Cooperation (TC) project (TAD5006) that aims to a) upgrade the existing capacities for the serological and molecular techniques for priority animal and zoonotic diseases in the country (Brucellosis, CSF, ASF, sheep pox, LSD, PPR, Rabies, and FMD), b) establish a bio-banking capacity and c) facilitate the implementation of the ISO 17025 standard in the local laboratories.

Two fellows received theoretical and practical training in serological techniques for detection of the priority diseases (basic agglutinations, precipitations, complement fixation, hemagglutination tests, agar gel immunodiffusion, as well as the various types of ELISA). An additional two fellows were trained in molecular detection and characterization of the priority pathogens, primarily via conventional and real-time PCR / RT-PCR and Sanger sequencing.



Serology training

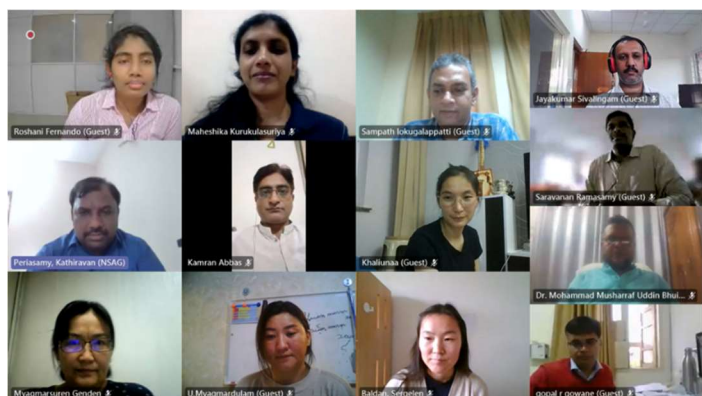
Both groups received training in basic organization of sample biobanking, data management and filtering records to identify sample location. The quality manager of the IVM was also trained in the implementation and maintenance of the quality management system ISO 17025.

Independently from the group fellowship, one additional scientist was trained at the IAEA Headquarters in Vienna in the use of the iVetNet information platform in data management needed for exchange and adaptation of validated Standard Operating Procedures (SOPs), quality assurance and quality control (QA/QC).

Virtual Regional Training on Bioinformatics data analysis for biodiversity and genome-wide association studies in livestock (MON5025)

As part of Mongolia's national Technical Cooperation project on Improving Breed Characterization of Cashmere Goats to Facilitate the Establishment of Strategic Breeding Programs (MON5025), a virtual regional training course was organized from 15 to 26 November 2021. The training course was attended by twenty-four participants from five countries (Bangladesh, India, Mongolia, Pakistan, Sri Lanka).

The course included lectures and practical hands-on training on: a) introduction to managing large sets of data using command line platforms: Basic Unix Commands, b) Introduction to 'R' and working with 'R' Studio, c) introduction to PLINK and data quality control (pruning genome wide single nucleotide polymorphic data, d) preparation of phenotype covariates and genotype data for GWAS, e) estimation of inbreeding and estimating effective population size using genomic data, f) assessment of population structure and estimation of genetic admixture.



Virtual regional training course on bioinformatics data analysis for Asian countries

Dr. Mario Barbato (Istituto di Zootecnia, Facoltà di Agraria, Università Cattolica del Sacro Cuore, Piacenza, Italy) served as an expert lecturer for the training course. The training was expected to help improve the regional capacity on handling large livestock genome datasets and utilizing genome wide information for biodiversity assessment, phenotype-genotype association studies, selection and breeding of local breeds for improved productivity.

Group Fellowship under the National TC Project in Bulgaria (BUL5017)

Ivancho Naletoski

Three scientists from partners in the national Technical Cooperation project (TC) in Bulgaria (BUL5017) were granted a group fellowship at the Croatian Veterinary Institute in Zagreb, under supervision of Dr Lorena Jemersic between 11 October and 5 November 2021.

The TC project integrates three institutions in one team for monitoring and control of Hepatitis E in the country. Those are the National Diagnostic Research Veterinary Medical Institute-NDRVMI (officially designated veterinary laboratory), the National Centre for Infectious and Parasitic Diseases (a public health laboratory), and the Faculty of Veterinary Medicine of University of Forestry, a multi-disciplinary educational institution.

Each counterpart institution's fellow was trained in the techniques used for virus isolation and serological and molecular detection of Hepatitis E. The fellowship also included practical training in the use of the APH Sanger Sequencing Service, data processing and phylogenetic analysis of viral isolates.



A fellow from the Faculty of Veterinary Medicine, Sofia, Dr Georgi Stoimenov, during the training in molecular techniques for detection of Hepatitis E virus

Fifth Research Coordination Meeting on Early Detection of Transboundary Animal Diseases to Facilitate Prevention and Control through a Veterinary Diagnostic Laboratory Network (VETLAB Network) (D32032)

Ivancho Naletoski

The fifth (final) Research Coordination Meeting of the Coordination Research Project D32032 was held online from 11 to 15 October 2021.

The purpose of the meeting was to focus on the produced secondary, serological and molecular standards for the priority diseases in the projects. Following secondary standards were produced by the partners in this CRP:

Avian influenza and Newcastle disease 310 panels PCR controls from each: H16, H5, H7, H9, Lasota, velogenic NDV. Each panel contains an amount sufficient for 10 test runs. Additionally, 160 vials antigen for the haemagglutination inhibition (HI) test for each H5, H7, H9 and NDV were produced. Each vial is sufficient for testing of 360 sera.

Rabies sera for Fluorescent Antibody Virus Neutralization (FAVN) test – 6100 ml negative and 3200 ml of positive serum.

Lumpy skin disease positive serum 4000 and 300 ml viral culture with a Ct value of ~20.

Sheep and goat pox positive serum (2000 ml) and viral culture (200 mL) with a Ct value ~17.

Brucellosis positive serum produced using *Brucella abortus* (1000 ml) and *Brucella melitensis* (1000 ml). Additionally, 300 positive aliquots of PCR DNA controls were produced for both, *Brucella abortus* and *Brucella melitensis* isolates, typed using MLVA-16 technique. Each of the aliquots, when diluted 1:1000 should have a Ct value ~30.

Peste des petits ruminants positive serum 2400 ml with competition percentage (sample to negative ratio) of ~12,5%. Approximately 2500 ml of viral culture for PCR was also produced with Ct of ~25.

African swine fever 2000 ml positive serum

Each of the produced standards will be transferred to the BSL-3 laboratory at the Austrian Agency for Health and Food Safety (AGES), Austria, for inactivation, lyophilization, re-titration and further dissemination among the laboratories of the VETLAB Network.

To facilitate the sustainable use of certified standards, the partners of the VETLAB Network will receive instructions and training on the production and verification of tertiary reference (national) standards, based on the existing international OIE (2021 a,b) and ISO (2006) guidelines and standards.

To facilitate the harmonization of diagnostic techniques, 73 validated SOPs (developed by the appropriate reference laboratories) were adapted to “ready-to-use” ISO format, and 13 additional SOPs were adapted in short format, as per the APH Laboratory template. All the SOPs were uploaded on the iVetNet Information Platform and are available for all registered counterparts of the APH sub-programme.



The fifth (final) Research Coordination Meeting of the CRP D32032

References:

ISO (2006). ISO Guide 35:2006 (revised by ISO Guide 35:2017). Reference materials — General and statistical principles for certification:
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OIE (2021a). International Reference Standards for Antigen Detection Assays:
<https://www.oie.int/app/uploads/2021/03/a-guideline-antigen-standards.pdf>

OIE (2021b) International Reference Antibody Standards for Antibody Assays:
https://www.oie.int/fileadmin/Home/eng/Our_scientific_expertise/docs/pdf/GUIDELINE_3_REF_STANDARDS_ANG.pdf

Coordination Meeting of the Veterinary Diagnostic Laboratory Network with Directors of African and Asian Veterinary Laboratories

Charles Lamien and Giovani Cattoli

The fourth coordination meeting of the Veterinary Diagnostic Laboratory Network (VETLAB Network) took place virtually from 11 to 15 October 2021. The meeting was attended by the twenty-two directors of the VETLAB partner laboratories from Bangladesh, Botswana, Burkina Faso, Chad, Côte d'Ivoire, Ethiopia (2 participants), Ghana, Kenya, Lao P.D.R, Lesotho, Eswatini, Morocco, Mozambique, Myanmar, Namibia, Nepal, Senegal, Sri Lanka, Thailand (2 participants), Tunisia, United Republic of Tanzania and Zambia. As in previous years, the directors of the VETLAB laboratories shared parts of the meeting with the VETLAB Research Coordination Meeting (RCM) participants.

The objectives of the meeting were as follows:

- a) Update on the VETLAB network activities, the VETLAB Coordinated Research Project (CRP) activities and other IAEA initiatives on transboundary animal diseases (TADs) and zoonoses,
- b) Discussing the 2021-2022 joint and individual country plans,
- c) Discussing among the VETLAB partner laboratories the challenges, priorities and the ways to improve laboratory diagnostics and surveillance for major TADs and zoonoses,
- d) Updating participants on the VETLAB CRP activities,
- e) Facilitating the exchange of experience, knowledge, and information between the Asian and African laboratories, and
- f) Discussing among VETLAB partner laboratories the ways to enhance collaborative research on laboratory detection and surveillance of major TADs and zoonoses.

The VETLAB partners presented their activities, achievements, challenges, and priorities, thus providing an essential reference for formulating individual and common work plans for implementing VETLAB network actions. The majority of the VETLAB partners acknowledged the essential role the VETLAB network plays in enabling them to establish and implement new molecular tests to detect various endemic and emerging TADs and zoonoses. Another outcome of the session was recognition that the countries share common challenges such as the procurement of appropriate reagents, the maintenance and calibration of essential laboratory equipment, the need for highly skilled personnel, the need to sustain or implement quality systems, and biosafety management programs. This led the participants to express encouragement to the VETLAB network to continue supporting the training of personnel in laboratory techniques, quality systems, and biosafety management. Additionally, they have endorsed the effort of the CRP to produce secondary reference material, which will facilitate the validation of new assays and support the implementation of quality systems.

Further on, it was recognized how important the yearly proficiency testing organized by the APH Laboratory is to the VETLAB partners. In the light of this recognition the VETLAB network is encouraged to expand such support by including new diseases for the proficiency testing and promote the provision of proficiency testing by other partners.

The APH Laboratory's presentation on the plan to support VETLAB partners on antimicrobial resistance related issues was received well. Last but not least, based on the successes observed in previous years, the voices gathered at the meeting encouraged the enhancement of collaborative research work within the network.

Such collaborative research could focus on priority TADs and zoonoses (i.e., LSDV, RVF, AI, NWD, ASF, rabies, brucellosis) or those relevant to each country and region.

National Training Course on Analyzing Disease Data with Priority on Hepatitis E using Modern Geo- Information (GIS) Systems (BUL5017)

Ivancho Naletoski

The virtual national training course in geographical information system (GIS) applications in disease monitoring and control with a focus on Hepatitis E, the target disease in the project BUL5017, was organized upon the request of the counterparts from Bulgaria, between 22 and 26 November 2021.

The aim of the course was to provide information and practical exercises on how to use rapidly evolving GIS software that has a role in decision making processes surrounding the control over the spread of animal diseases. GIS software offers user friendly solutions for large and multi-source datasets adjusted to the skills of non-professionals users.

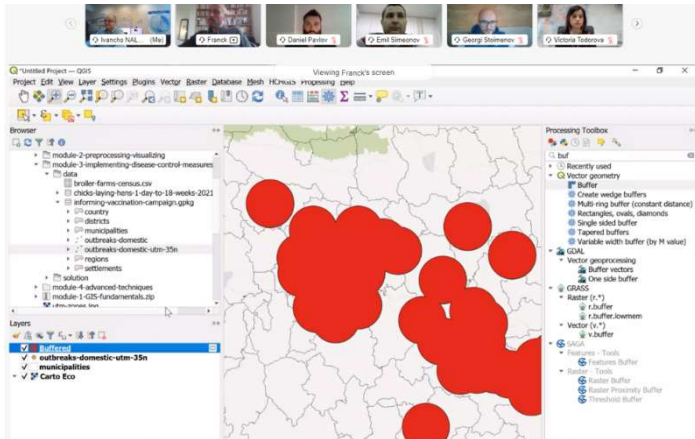
Usually, after samples from the field are tested for the presence of one or more diseases (& other parameters), the testing reports are submitted to the veterinary authority (head veterinary office / chief veterinary officer - CVO). The CVO office reviews and analyzes the data received, allowing for rapid decision-making on the actions to curb the spread of the disease or/and minimize the impact of the spread. The decision-making process is relatively straightforward when analyzing small datasets. When large datasets are in play, especially if they are continually provided over a longer period, the analysis and decision making becomes a complicated and cumbersome process.

To cope with the complexity, specialized GIS software enables the linking of geo-referenced tabular datasets for their visualization directly on the maps in real time. Moreover, by merging multiple datasets into multiple GIS layers, it becomes possible to determine the influence that various risk factors have on the disease spread and thus rapidly deploy the most effective measures to curb them.

For example, GIS software allows users to combine the animal census data (1st layer) with the vaccination status (2nd layer) and detected positive animals (3rd layer) over a certain geographical area and estimate the risk for further spread of the disease.

Additional datasets such as animal movements, processing plants for animal products, meteorology, etc. may also be included to support the decision-making process.

The course was supervised by a GIS expert lecturer, Mr Franck Albinet from France. Among the covered topics were: coordinate reference systems; use of multiple layers in GIS analysis; linking tabular datasets to GIS; presentation of basic statistical parameters on the map (data aggregation techniques); monitoring disease development and buffer zones, and monitoring disease control programmes (vaccinations) using GIS.



Screenshot of the virtual event

Training Course for Veterinary Diagnostic Laboratory Network Partners on Sequencing and Bioinformatics

Charles Lamien

The training course for Veterinary Diagnostic Laboratory Network Partners on Sequencing and Bioinformatics took place virtually from 29 November to 10 December 2021. The aim of the training course was to provide basic knowledge on next-generation sequencing (NGS) and NGS data analysis on the Linux interface. In addition, the participants received training on advanced concepts of the phylogenetic analysis of viruses.

The two-week training course was attended by eighteen participants from nine countries (Bangladesh, Botswana, Ethiopia, Indonesia, Malaysia, Morocco, Senegal, Thailand, Tunisia). Prior to the training course, the participants were assisted on a bilateral level with the set-up of a virtual Linux machine, which enabled them to run hands-on exercises on provided datasets.

Group Delivery of Diagnostic Packages for Early Detection of Zoonotic Diseases at the Animal-to-Human Interface (INT5157 – Pillar 1 of the ZODIAC Initiative)

Ivancho Naletoski

The objective of the Pillar 1 of the ZODIAC Initiative (TC Project INT5157) is to upgrade the capacities of the nominated ZODIAC National Laboratories (ZNLs) in the early detection and mining of zoonotic pathogens at the animal-to-human interface. Considering that ZODIAC is an off-cycle initiative, based on extra-budgetary contributions, the delivery of diagnostic packages is organized in phases.

The diagnostic package comprises

- core equipment and accessories for detection of specific antibodies against the priority zoonotic diseases, and
- core equipment for molecular detection of the causative pathogens. Certain ZNLs, those with experience and history in the use of Sanger sequencing, were selected for installation of a next generation sequencing (NGS) package and are foreseen as training centers for the regions.

Parallel to the delivery of the diagnostic packages, the IAEA in collaboration with FAO is organizing training courses for selected priority diseases specific to each of the geographical regions. For each of the planned training courses, expert teams will be invited to harmonize the training programmes and support the hands-on practical exercises.

Phase One was finalized with the equipment and accessories defined, the packages ordered and delivery programmed. Phase Two is currently under preparation (please see the map).



Phase one recipient laboratories indicated in blue. Phase two recipient laboratories (tentatively) indicated in red. Darker color fill (blue or red) indicates laboratories to receive the conventional diagnostic package, lighter color fill (light blue or light red) indicates selection for installation of the NGS packages

In Phase One, 20 laboratories (7: Africa, 2: Asia, 5: Europe, 6: Latin America) were selected for the classical diagnostic package and 5 laboratories (Botswana, Senegal, Tunisia, Indonesia and Thailand) for the NGS package.

In Phase Two (tentatively), 16 laboratories will be selected (8: Africa, 5: Asia and Europe, 3: Latin America).

The tentative selection of the laboratories for the NGS packages comprises Portugal, Slovakia, Argentina and Brazil. The recipient laboratories of the NGS packages will also receive a comprehensive “training-of-trainers” programme, in order to transfer “hands-on” experience of standardized NGS procedures, and simultaneously prepare them to train others.

Group Delivery of Equipment and Consumables for Periodical Verification of Biosafety Cabinets (RAF5082)

Ivancho Naletoski

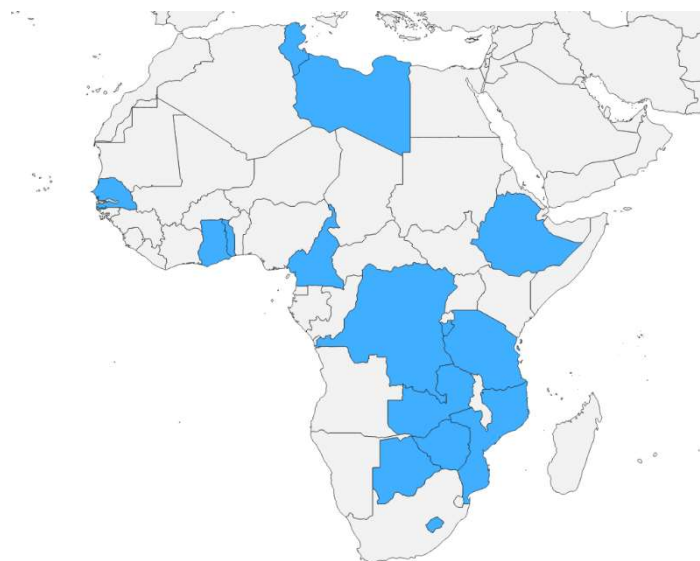
Biosafety cabinets (BSCs) are critical devices in the biosafety of a laboratory, especially when dealing with highly contagious infectious diseases. For proper functioning they need periodical (usually annual) verification and calibration, as well as regular replacement of the microbiological filters.

Failures in the periodical maintenance of the BSCs may result in the escape of dangerous pathogens from the designated laboratory area and/or laboratory facilities. The former may result in infected staff (in case of zoonotic diseases); the later can have unprecedented consequences on a larger scale.

Many counterparts' laboratories, especially in the African regions, have either no access to authorized maintenance services or do not have sufficient funds to cover the regular maintenance costs. The estimation of the annual costs for an external service provider are between 30% and 50% of the cost of a new biosafety cabinet.

To provide a solution to the above-mentioned issues, under the ongoing Technical Cooperation project (TC) RAF5082, a training programme on installation, maintenance, and calibration of BSCs with certification will be organized in the 1st quarter of 2022.

The necessary equipment and consumables for local application were delivered before the training course to the selected counterparts' laboratories, compiled by the IAEA in collaboration with the team of expert lecturers. By passing the theoretical and practical exams, the successful participants are authorized to perform verification and calibration to all the BSCs in their own laboratories.



Map of Africa showing the recipient laboratories (countries in blue) which are planned also for the training course with certification

The training course covers all quality aspects, including installation of BSCs (laminar flow, BSCs, fumehoods, ductless fume cabinets, PCR cabinets and others), introduction to the test equipment used, siting requirements, inflow and downflow testing, filter integrity testing, flow visualization testing, site assessment testing, and user comfort testing – (light test and noise test).

Regional Training Courses on Selected Topics of Biosafety in Veterinary Laboratories (RAS5085 and RER5025) – virtual events

Ivancho Naletoski

A set of eleven online training courses on the basic principles of biosafety in multiple activities performed in the veterinary laboratories was organized under the ongoing Technical Cooperation projects RAS5085 (Member States (MSs) of the IAEA in Asia) and RER5025 (MSs of the IAEA in Europe), between May and December 2021.

The following courses were included in the training sessions: a) biological risk assessment – How safe are we in our labs if we apply the risk-based approach according to the new WHO Biosafety Manual, b) ISO 35001 – An introduction to the biorisk management standard, c) gene drives: technologies, applications & biosafety challenges; d) introduction to vaccinology, e) auditing for maximum impact – an introduction, f) Disinfection and sterilization; g) bloodborne viruses and pathogens, h) biosafety culture, i) epidemiology - what is it and what do these numbers really mean, j) personal protective equipment, and k) blended learning.

The trainings were held by a range of experts from the European Biosafety Association (EBSA). The courses were attended by 42 participants from 20 MSs in Asia, and 34 participants from 21 MSs in Europe (RER5025). Among the former were Bangladesh, Brunei, Cambodia, Fiji, Iran, Kuwait, Lebanon, Malaysia, Mongolia, Myanmar, Nepal, Oman, Pakistan, Palestine, Papua New Guinea, Philippines, Qatar, Syria, Thailand and the United Arab Emirates. Among the latter were Albania, Azerbaijan, Belarus, Bosnia and Herzegovina, Bulgaria, Croatia, Cyprus, Estonia, Greece, Kyrgyzstan, Latvia, Lithuania, Moldova, Montenegro, North Macedonia, Romania, Serbia, Slovenia, Tajikistan, Turkey and Uzbekistan.

All participants have received a one-year membership to EBSA, which will enable them access to international standards, webinars, workshops and training courses available online on the restricted part of the EBSA website.

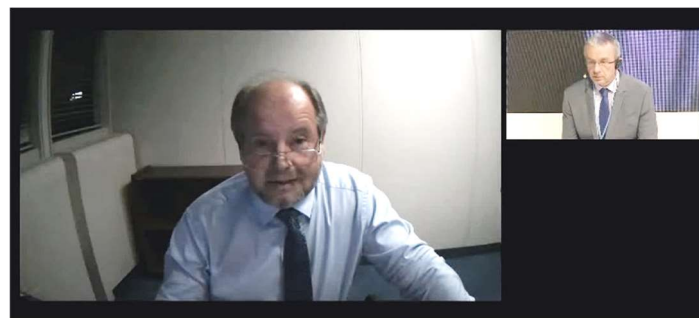
Stories

Nuclear Science Helps to Adapt to Climate Change, COP26 Participants Hear

More intensive droughts and floods, recurring wildfires, dangerous pest and crop diseases - nuclear science and technology are helping countries and communities to adapt to and cope with climate impacts. International experts at a side event of the 26th United Nations Climate Change Conference (COP26) highlighted how this works.

The event, organized by the IAEA and titled Contribution of Nuclear Science and Technology to Climate Adaptation, was organized on 6 November as one of the COP26 nature and land-use side events. It demonstrated how governments, farmers and others can increase resilience to the impacts of climate change and achieve more sustainable management of land and water by protecting and restoring nature and reforming the food and farming systems by using nuclear science and technology.

Experts discussed the ability of nuclear and related techniques in boosting agricultural resilience to climate change, in reducing greenhouse gas emissions, and in increasing agricultural productivity – altogether known as climate-smart agriculture. Norbert Nowotny of the Joint FAO/IAEA Centre gave a presentation on the connection between the zoonotic diseases and climate.



Norbert Nowotny, APH, giving a talk at the COP26 side event organized by the IAEA on November 6, 2021

This event raised awareness about the role of nuclear science and technology in climate smart agriculture, and in climate adaptation overall. The event highlighted the support the IAEA provides to countries in relation to climate adaptation and monitoring, including capacity building, research and the transfer of equipment.

“With the power of atoms, we have the tools to increase our resilience to the global change,” said IAEA Director General Rafael Mariano Grossi, following the release of an IAEA report setting out how nuclear techniques can help the world adapt to a changing climate and the increasing frequency of extreme weather events.

“The IAEA is here to help countries and farmers establish climate-smart agriculture practices, improve food security, locate groundwater, understand the impact of global warming on zoonotic diseases, and fight pests like the mosquito and fruit fly,” Mr Grossi said, opening the event. “We turn nuclear science and technology into climate action.”

[Click here](#) to read more

[Click here](#) to watch the video

What is a zoonotic disease?

Zoonotic diseases are infectious diseases that are transmitted from animals to humans, like COVID-19, bird flu, malaria or Ebola. Zoonotic diseases affect around 2.6 billion people every year. Some zoonotic diseases, such as rabies, only spread from direct animal-to-human contact.



Others, more dangerously, start with animals and are capable of causing widespread human-to-human transmission. Nuclear-derived techniques can be used to track pathogens as they move from animals to humans and thus help the world respond better to any future outbreaks.

*What is a zoonotic disease?,
an animation video*

[Click here](#) to watch the video

NEW CRP: Nuclear and related techniques to measure the impact of type of feeding and production system on greenhouse gas (GHG) emissions and livestock productivity (D31031)

Livestock are fundamental for sustainable diets and livelihoods, especially in developing countries, providing much needed nutrients for healthy households as well as income from sale of animal and animal products. However, livestock keeping faces many challenges including availability of adequate quantity and quality feed, in addition to contributing to climate change. Global warming has occurred as greenhouse gases (GHG) accumulate in the atmosphere. Global livestock agriculture has been cited to be responsible for up to 14% of anthropogenic GHG emissions annually. Greenhouse gas emission from livestock production is influenced by a number of factors, including diet composition and digestibility. Nutrition and feeding strategies may be able to reduce GHG emissions intensities by up to 15%. Better balancing of key nutrients in the diet would increase digestive efficiency and the help reduce the carbon footprint of animal products.

The IAEA is launching a 5-year Coordinated Research Project (CRP) on applying nuclear and related technologies and resources to optimize livestock feeding practices to reduce GHG emissions and help mitigate climate change. Dairy cattle production systems will be targeted. The CRP will evaluate nitrogen and energy supplementation strategies in cattle feeding to mitigate enteric and manure GHG emissions, develop and/or validate nuclear and related tools/resources for nutrition-based GHG emission reduction in cattle production, and recommend tools and mechanisms to monitor livestock GHG emissions.

The CRP will involve 10 Research Contract (RC) holders from developing countries, three Technical Contract (TC) holders and four Research Agreement (RA) holders from laboratories engaged in high-level livestock GHG emission and mitigation research. Nuclear techniques involving compound-specific stable isotope (CSSI) of ^{15}N will be utilized to address the objectives of the CRP.

CRP Overall Objective

The overall objective is to enable IAEA member states, especially developing countries, to use nuclear and related technologies and resources to optimize livestock feeding practices that reduce GHG emissions and help mitigate climate change.

Specific Research Objectives

- To strengthen capacity in developing countries in the use of established or novel methodologies to determine seasonal changes in forage/feed quality, biomass, and intake.
- To identify locally-available non-human edible feeds, including agro-industrial by-products from food systems, for nitrogen and energy supplementation in cattle feeding.
- To evaluate nitrogen and energy supplementation options in cattle feeding to mitigate enteric and manure GHG emission.
- To develop and/or validate nuclear and related tools/resources for nutrition related GHG mitigation in cattle production.
- To provide perspective on applying GHG mitigation strategies to support cattle feeding decisions.

How to join this CRP

Please submit your Proposal for Research Contract or Agreement by email, to the IAEA's [Research Contracts Administration Section](#), using the appropriate template on the [CRA](#) web portal. Note that the same template can be used for both research and/or technical contracts.

For further information related to this CRP, potential applicants should use the [contact form](#) under the CRP page.

The Joint FAO/IAEA Centre honoured by the IAEA “One-House” Superior Achievement Award 2021 for its COVID-19 Support Actions

For more than 50 years, the FAO and the IAEA have been expanding knowledge and enhancing capacity in leveraging nuclear sciences to help feed the world and recently strengthened their partnership, creating a Joint FAO/IAEA Centre of Nuclear Techniques in Food and Agriculture.

In the IAEA **Superior Achievement Award** ceremony held on 3 December 2021, the Joint FAO/IAEA Centre of the Nuclear Applications and Techniques Department together with IAEA colleagues from two other departments (Technical Cooperation and Management) were recognized for assisting IAEA Member States in the early detection of SARS-CoV2 and the control of COVID-19. Under the motto “One-House for One-Health”, the IAEA ‘One-House’ award 2021 underlines how effective teamwork can support the timely detection, diagnoses, and control of animal and zoonotic disease outbreaks.

Building on the longstanding experience of the Animal Production and Health Section (APH) of the Joint FAO/IAEA Centre with transboundary animal and zoonotic disease outbreaks, the Centre, through its Animal Production and Health Laboratory and VETLAB Network, has been closely cooperating with veterinary laboratories in the field to assist them with the COVID-19 outbreak. This was the largest technical support initiative since the agency's foundation amounting over 36 million euros to fight the pandemic. Among the achievements were:

- The Agency provided COVID-19 emergency packages to 129 IAEA Member States with full technical advice and backup from the Joint FAO/IAEA Centre.
- The Agency provided guidance and expert services to 305 medical and veterinary laboratories involved on COVID-19 testing.
- The Joint FAO/IAEA Centre, through its Animal Production and Health Laboratory and VETLAB Network, provided timely guidance and validated technical procedures on COVID-19 detection to 124 veterinary laboratories in 46 Member States.
- The Joint FAO/IAEA Centre provided direct one-on-one technical back stopping to 87 veterinary laboratories.

Further to the IAEA's direct COVID-19 response, the IAEA launched the ZODIAC (Zoonotic Disease Integrated Action) project to assist Member States to be better prepared for future pandemics and outbreaks of zoonotic diseases.

The Animal production and Health Section of the Joint FAO/IAEA Centre, with its expertise in nuclear science and its applications in animal health and through the cooperation with the FAO, assists Member States in building their technical capacities to prepare for and respond to threats and outbreaks of transboundary animal and zoonotic diseases.



The Animal Production and Health Team of the Joint FAO/IAEA Centre and the IAEA Director General (DG) at the Award Ceremony (from left to right): Mr Ivancho Naletoski (FAO), DG Rafael Mariano Grossi, Mr Gerrit Viljoen (IAEA), Mr Charles Lamien (FAO) and Mr Giovanni Cattoli (IAEA)

Research Activities of the Animal Production and Health Laboratory

Animal Health

Assessing D10 Values of two African Swine Fever Virus Strains to Determine Optimal Irradiation Dose for Vaccine Antigen Candidates

In the pursuit of producing an efficacious vaccine against African swine fever (ASF) virus, the APH Laboratory, in collaboration with the Friedrich Loeffler Institut (FLI, Germany), has started a project to produce and test in vivo a gamma-inactivated vaccine. As of today, all the attempts made to produce an efficacious inactivated vaccine against ASF virus, from chemical to heat inactivated, have failed their purpose. So far, only Live Attenuated Vaccines (LAV) have proven to be efficacious, albeit with limited success. However, this type of vaccine has safety concerns of reverting back to its infectious status, thus posing a threat of new outbreaks and even the appearance of new variants. Therefore, the ideal vaccine against ASF virus would be a fully inactivated one, able to ensure safety while conferring protection against lethal challenge.

Neither chemical nor heat-inactivated tested inactivated vaccine candidates have been able to elicit an immune response strong enough to confer protection to the host. However, the technology based on gamma-radiation is able to completely inactivate a pathogen while preserving the antigenicity and structural integrity. In simple terms, this peculiarity leads the immune system of the host to recognize the pathogen as if it were live and thus mount a successful immune response. Nevertheless, the variability among different types of pathogens, whether they are viruses, bacteria or parasites, and factors such as type of nucleic acid and composition of outer structures imply that gamma radiation, and thus vaccine composition, needs to be fine-tuned according to the characteristics of the pathogen. The optimization consists of understanding at what temperature of irradiation, what radio- or cryo-protectant compound to be used and the dose of irradiation needed, among other factors.

APH Laboratory has standardized some steps of the optimizing process: we irradiate at frozen conditions (-80°C) and we use trehalose as cryo-protectant. To determine the ideal gamma-dose, the required step is to assess the D10 value of the pathogen. D10 is defined as the ability of gamma irradiation to reduce an exposed microbial population by 90 per cent (one log₁₀) under standard

conditions of time, temperature and dose. By knowing the D10 value and the virus-titer of the batch, it is possible to calculate the optimal minimum inactivation dose for the amount of pathogen in the vaccine preparation.

For our purpose of producing a gamma-inactivated vaccine against ASF virus, two different strains of ASF virus, Genotype II Estonia2014 and Armenia08, were irradiated at different doses (0, 2, 4, 5, 6, 7 and 8 kGy) in order to assess the D10 value. Based on the virus titers recorded by the hemadsorption assay after each irradiation dose, it was possible to calculate the D10 value for each strain as it shown in Figure 1.

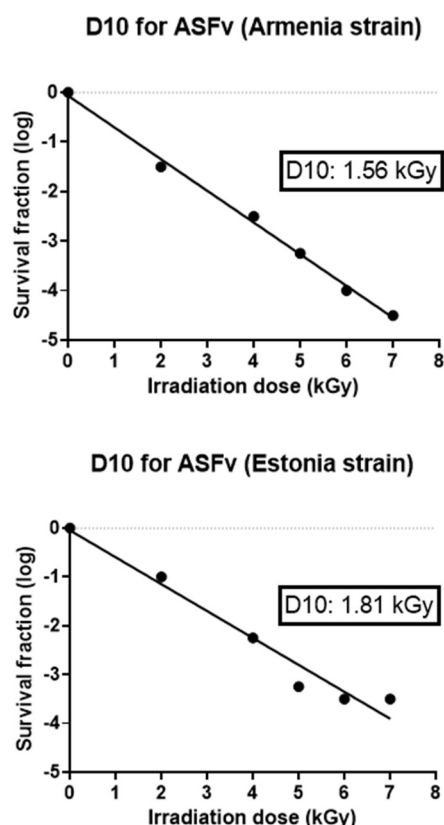


Figure 1: D10 values calculated for Armenia and Estonia strains

By knowing the titer of the non-irradiated batches, which was 105.25 HAD₅₀/ml, we were able to calculate the optimal minimum irradiation dose for these two strains. These were 8.2 kGy for Armenia and 9.5 kGy for Estonia, which were rounded up to 10 kGy. In addition to this, to ensure a further safety layer, a Sterility assurance level (SAL) was applied. SAL is a given probability that any single pathogen within a sample may escape inactivation following an exposure to γ -irradiation. Here at the IAEA, we recommend a SAL of 10^{-6} which means that there is a one in a million chance of a single infectious particle remaining following irradiation.

Thus, applying a SAL in addition to the calculated optimal minimum dose, we were able to establish that the optimal gamma inactivation dose required to produce a vaccine against ASF virus using the two strains tested is 20 kGy.

Production of Antibodies for the Development of a Bovine IgA- ELISA to Quantify Mucosal Immune Response

Most pathogens enter their hosts through mucosal surfaces. Mucosal surfaces are in organs that contain mucous membranes, such as those present in the respiratory, digestive, and urogenital systems. This also includes eye conjunctiva, the inner ear, and the ducts of all exocrine glands. Because these surfaces encounter pathogens more frequently, they have developed a special kind of protective mechanism.

Therefore, targeting mucosal immunity is a very attractive strategy in vaccine development. It is well known that delivery of vaccine antigens at the mucosal surface leads to protection at the vaccinated mucosal surface and in connected mucosal tissues as well. Since the induced immunity is at the port of entry, the infection could be prevented at a very early stage. Logistically, mucosal vaccines are also a very attractive approach because of ease of application i.e. in the form of aerosols or through drinking. The hallmark of the effector mechanisms for mucosal immune response includes the secretory IgA (SIgA), a protease-resistant antibody and the cell-mediated mucosal immune response. These effectors have been shown to be capable of the clearance of various pathogens including enteric/respiratory viruses and intracellular parasites. ELISA is a technique used to quantify IgA antibodies.

The APH Laboratory has initiated a project to develop an indirect ELISA to measure pathogen specific IgA in cattle. In the first step of this project, a hybridoma cell line (IL-A71, kindly donated by Dr Jan Naessens of the International Livestock Research Institute) that produces anti-bovine IgA antibodies were expanded and antibodies were produced in the T-Flask system or by the bioreactor semi-permeable system. Next, using Protein G affinity chromatography columns, the produced antibodies were purified. Through PAGE-gel electrophoresis the purity of concentrated antibodies was confirmed (Figure 2). Purified antibodies were then conjugated with horseradish peroxidase enzyme (HRP). Finally, the function of antibodies was assessed through their ability to recognize bovine IgA.

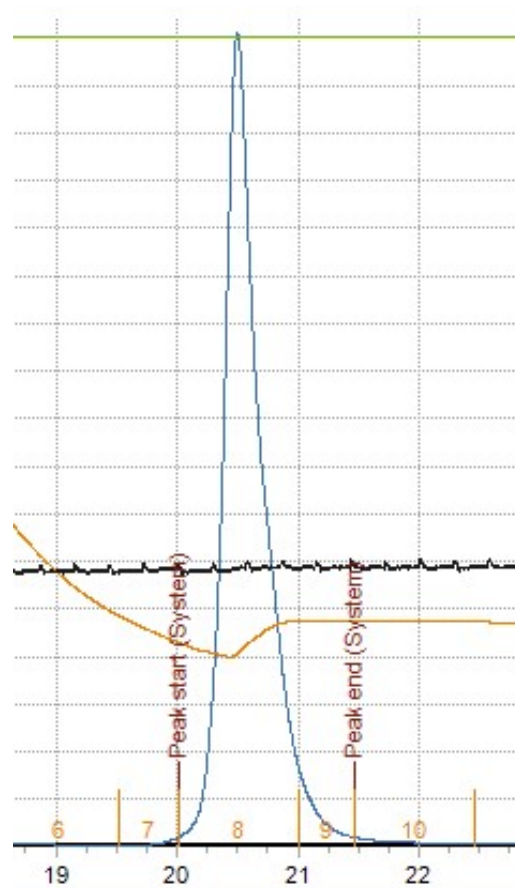
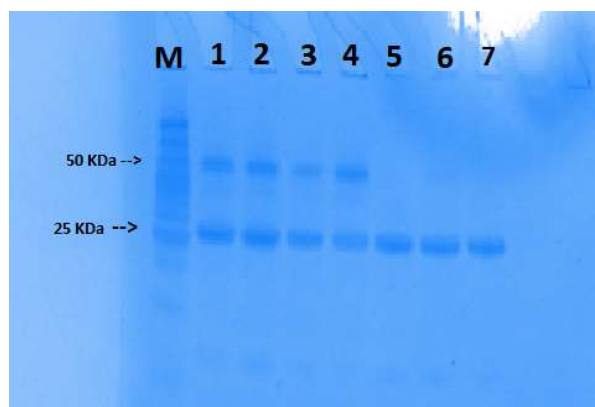


Figure 2: Purification of mouse anti-bovine IgA produced by IL-71 cell line

Above: Shows elution of concentrated antibodies which was bind to protein G affinity chromatography columns as a UV signal (blue line)

Left: Representative PAGE gel images showing the heavy chain (50 KDa) and light chain (25 KDa) of the purified antibodies (M: marker lane and 1-4 are various samples obtained through bioreactor system and T-flasks)

Immunomodulation Properties of Irradiated Lactobacilli

Probiotics have been extensively studied for their broad application in human and animal health, thanks to their ability to stimulate the immune system of the host. This characteristic is one of the multiple health benefits that these microorganisms are capable to confer to the host organism. This range of effects on the host strictly depends on various factors including the type of strain selected, dose used and time of administration.

Recently, there has been an increasing interest in producing inactivated probiotics, also known as “para-probiotics”. The reason behind this interest is the acknowledgement of increasing safety concerns regarding the application of these products on immune-compromised individuals and in neonates. In fact, there is evidence of translocation of gut bacteria into the circulation (leading in some cases to infection), alteration of natural microbiota and transfer of genes from probiotic bacteria to commensals and pathogens leading to anti-microbial resistance.

A literature review on the possible activity of non-viable probiotics concluded that certain probiotic effects, such as immune modulation, can also be obtained with inactivated probiotics. Furthermore, it was demonstrated that completely opposite immunological results can occur based on the mode of inactivation chosen.

Heat and irradiation treatments differ in their mode of inactivation of a microorganism. When bacteria are exposed to increasing temperature, significant changes occur in protein components of the cells. Initially, as the temperature increases, the bonds in the protein molecules are weakened and at higher temperature they are broken. There is a loss of enzymatic activity and decrease in the solubility of the proteins. At high enough temperature, proteins will denature and coagulate, causing cell death. It may be that due to heat, these denatured proteins may contribute to an enhanced immune-stimulation.

Irradiation, on the other hand, destroys microorganisms without appreciably raising the temperature. In our case, the high-energy gamma rays generated by the excited nucleus of Cobalt 60 altered the DNA of the bacteria either by breaking molecular bonds or by polymerization of the DNA, resulting in the death of the cell. Since irradiation does not alter the proteins or other components of the cell, it could be predicted that this type of inactivation would yield a product more similar to the live version.

To understand more about this, a collaboration between University of Natural Resources and Life Sciences (BOKU) of Vienna and the APH Laboratory was established. BOKU provided four different strains of Lactobacilli, which are among the most studied type of probiotics, namely *L. casei*, *L. acidophilus*, *L. paracasei* and *L. plantarum*.

Several experiments were conducted to understand the differences between the four strains tested and moreover, how heat- and gamma irradiation-treatment modify the characteristics of the bacteria.

First, the D10 value of each strain was determined. This is the dose of gamma irradiation needed to lower the concentration of an organism by one log10. Then, the metabolic activity was analyzed, demonstrating how irradiated inactivated Lactobacilli were able to preserve the ATP production, membrane and redox potential compared to heat-treated ones. Finally, to assess immune-stimulatory capacity, peripheral blood mononuclear cells (PBMCs), isolated from fresh blood collected from five sows, were separately incubated in vitro for 16 hours with each of the four strains in each of the versions tested (live, heat-treated and gamma-irradiated).

Following these steps, RT-PCR analysis evaluating the expression of 26 different immune markers targeting adaptive and cell-mediated immune response cytokines, pathogen-recognition receptors and signal transduction pathways was performed.

From the results, a heat map analysis showed that *L. acidophilus* and *L. paracasei* when irradiated showed an overall gene expression behaviour similar to their live versions (Figure 3), whereas *L. casei* and *L. plantarum* showed a different trend.

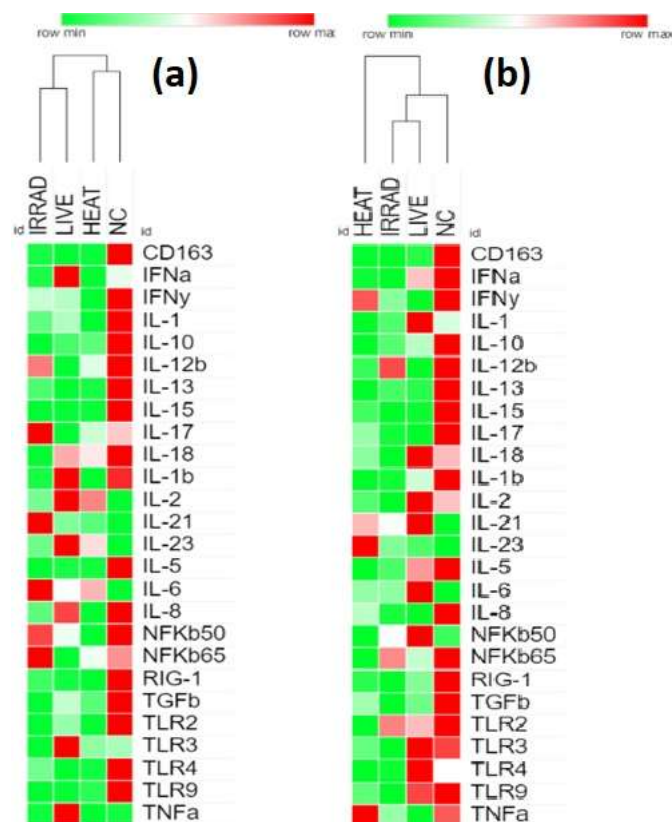


Figure 3: *L. acidophilus* (a) and *L. paracasei* (b) gene expression heat maps (IRRAD = irradiated, LIVE = non-treated, HEAT = heat treated, and NC = negative control)

According to a one-way ANOVA statistical analysis, the different strains with different treatments were able to up- or down-regulate significantly four pro-inflammatory cytokines (IFNa, IL-6, IL-21 and IL-23), one anti-inflammatory cytokine (TGFb) and one pathogen recognition receptor (TLR9), as shown in the examples in Figure 4.

Depending on the strain selected and the treatment used, the immune stimulation on PBMCs changed. This represents a powerful and useful tool whenever a selection of a vaccine adjuvant needs to be strategically made. Some strains show a better capacity to stimulate some key pro-inflammatory cytokines compared to others and the selection of heat- or gamma irradiated-treatment can boost or reduce this effect.

The next step will be an application in vivo of selected inactivated-Lactobacilli strains, to test their efficacy as potential vaccine adjuvants.

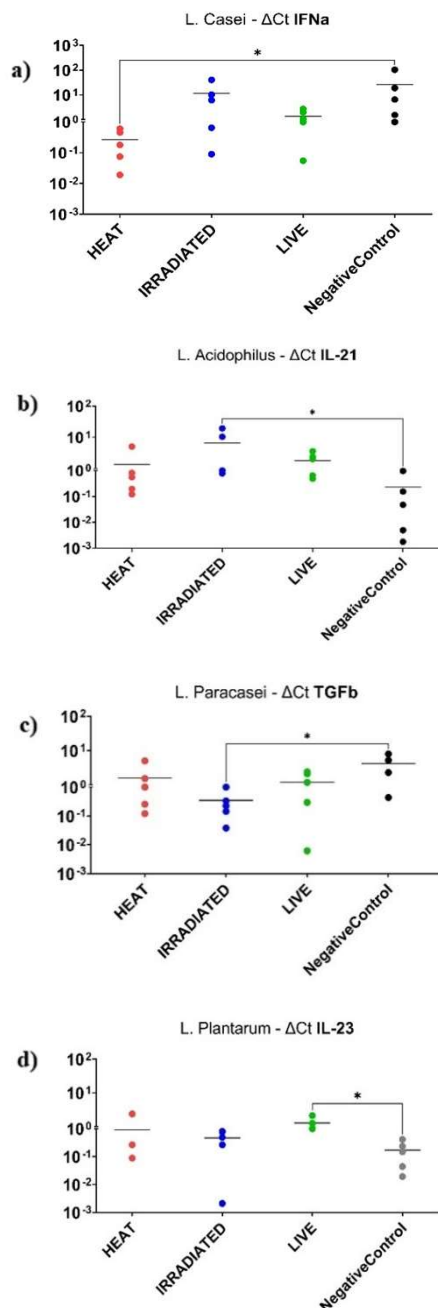


Figure 4: a) Heat-treated *L. Casei* induce down-regulation of IFNα; b) Gamma-irradiated *L. Acidophilus* induce up-regulation of IL-21; c) Gamma-irradiated *L. Paracasei* induce down-regulation of TGFβ; d) Live *L. Plantarum* induce up-regulation of IL-23

Evaluation of Real-Time PCR Based Detection Kits for SARS-Cov-2

Mitigation of SARS-CoV-2 transmission requires the availability of accurate and sensitive detection methods. In low-resource settings, the cost and availability of commercial kits availability can limit many diagnostic laboratories. In such cases, laboratories need to identify alternative and cheaper reagents.

The APH Laboratory, in collaboration with the Austrian Agency for Health and Food Safety (AGES, Austria), evaluated eight commercial qPCR ready mixes from Applied Biosystems, Bio-Rad, Biotech Rabbit, Invitrogen,

Promega, Qiagen, QuantaBio, and Takara and three ad hoc molecular diagnostic kits GeneFinder (Osang Healthcare), Genesig (Primerdesign), and Vioreal (Ingenetix). The limit of detection for each assay was determined by using serial dilutions of a defined clinical sample. The clinical sensitivity was assessed against a panel of 178 clinical samples (Figure 5) and specificity against a panel of human beta coronaviruses.

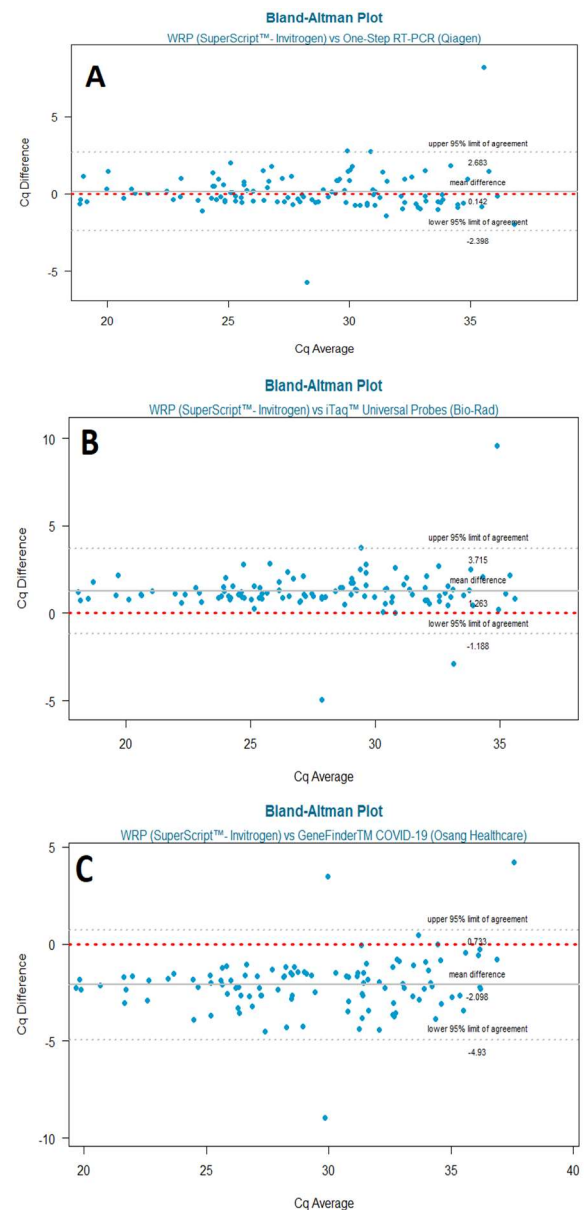


Figure 5: (A-B-C) Bland-Altman plots comparing the WRP to three representative kits. The plots show differences between the Cq values of the WRP and the tested kits against the average of the Cq values: **A.** WRP vs One-Step RT-PCR (Qiagen), showing an example of almost perfect agreement; **B.** WRP vs. iTaQ™ Universal Probes One-Step Kit (Bio-Rad), showing an example of a tested kit with lower Cq values than the WRP; **C.** WRP vs GeneFinder™ COVID-19 Plus RealAmp kit (Primerdesign Ltd.), showing an example of a tested kit with higher Cq values than the WRP

The red dotted lines represent the lines of identity (i.e. perfect agreement). The grey lines represent the bias between the test kits and the WRP. The grey dotted lines represent the limits (upper and lower) of agreements. Bland-Altman plots for all the tested products are illustrated in the supplemental material

The inter-assay agreement was determined using statistical tests (Bland-Altman, Fleiss-Kappa, and Cohen's Kappa) and was good to excellent in all cases. This study showed that all these assays are suitable for the routine detection of SARS-CoV-2.

Therefore, the qPCR Ready Mixes are a valid alternative to ad hoc molecular diagnostic kits. The findings were published in the Journal of Virological Methods (Dundon et al (2021). DOI: 10.1016/j.jviromet.2021.114200).

Poxviruses in Cattle, Sheep, and Goats in Botswana

Even though Lumpy skin disease (LSD) has been observed in Botswana since 1943, following an outbreak in the north of the country in Ngamiland, poxvirus diseases in ruminants have received little attention. Activity has been especially scarce when it comes to the molecular characterization of the circulating viruses. Because several poxviruses belonging to the *Capripoxvirus*, *Orthopoxvirus*, and *Parapoxvirus* genera can infect livestock and induce similar clinical symptoms in common host species, the diagnosis is challenging, even for scientists in areas where LSD is endemic. To characterize poxviruses circulating in ruminants in Botswana, skin biopsy and skin scab samples from cattle, sheep, and goats were analyzed using a high-resolution melting (HRM) assay that detects and differentiates poxviruses. *Capripoxviruses* and *Parapoxviruses* were further characterized by sequence analysis of RPO30 and GPCR genes and the B2L gene.

The HRM assay revealed lumpy skin disease (LSD) virus in three cattle samples, pseudocowpox (PCP) virus in one cattle sample, and orf virus (ORF) in one goat and one sheep sample. The phylogenetic analyses, based on the RPO30 (Figure 6) and GPCR multiple sequence alignments, showed that the LSD virus sequences of Botswana were similar to common LSD virus field isolates encountered in Africa, Asia, and Europe. The Botswana PCP virus presented unique features and clustered between camel and cattle PCP virus isolates. The Botswana ORF virus sequence isolated from goats differed from the ORF virus sequence isolated from sheep.

This study is the first report on the genetic characterization of poxvirus diseases circulating in Botswana cattle, goats, and sheep, which has confirmed pseudocowpox in the country and provided the first molecular characterization of LSDV ORFV and PCPV. These findings were published in the Virology Journal (Modise et al (2021). DOI:10.1186/s12985-021-01634-9).

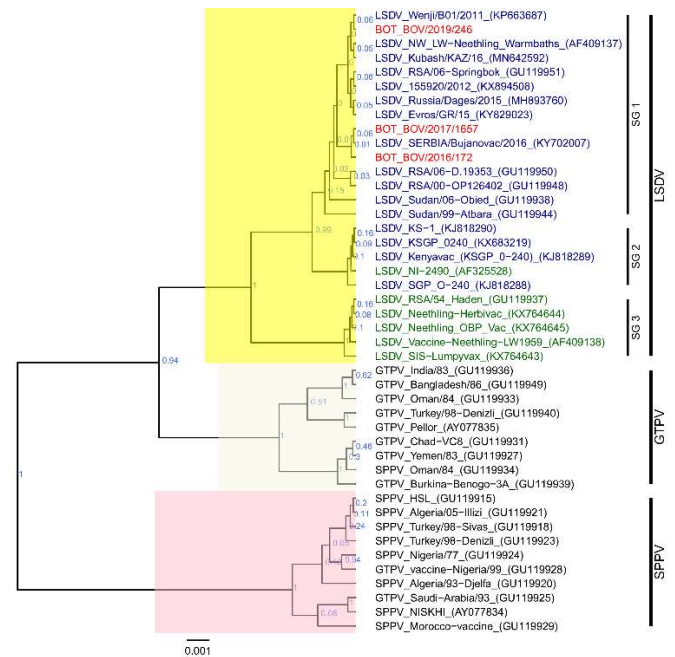


Figure 6: Maximum clade credibility tree based on the complete RPO30 complete gene sequences of capripoxviruses. The posterior probabilities are plotted as respective nodes labels. LSDVs from Botswana are highlighted in red

SARS-CoV-2 for Antibody Detection in Different Animal Species by Using Luciferase Immunoprecipitation System (LIPS)

The recent emergence of SARS-CoV-2 in humans from a yet unidentified animal reservoir and the capacity of the virus to naturally infect pets and farmed animals, and potentially wild animals, has highlighted the need for serological surveillance tools.

The APH Laboratory has, in collaboration with several partners (National Institutes of Health, Bethesda, Maryland, USA; Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro, Italy, and Friedrich-Loeffler-Institut; Greifswald-Insel Riems, Germany) evaluated the suitability of the luciferase immunoprecipitation system (LIPS) technology employing the Spike (S) and Nucleocapsid proteins (N) of SARS-CoV-2 for antibody detection in different animal species.

Sera from SARS-CoV-2 naturally infected mink (n=77), experimentally SARS-CoV-2-infected ferrets, fruit bats, and hamsters, and a rabbit vaccinated with a purified spike protein, were examined for antibodies by using the SARS-CoV-2 nucleocapsid (N) and/or spike (S) proteins. From comparison with known neutralization status of the serum samples, statistical analyses, including calculation of the Spearman rank-order-correlation coefficient and Cohen's kappa agreement, were used to interpret the antibody results and diagnostic performance.

The LIPS immunoassay robustly detected the presence of viral antibodies in naturally infected SARS-CoV-2 mink and experimentally infected ferrets, fruit bats, and hamsters. For the SARS-CoV-2-LIPS-S assay, there was a good level of discrimination between the positive and negative samples for each of the five species tested with 100% agreement with the virus neutralization results. In contrast, the SARS-CoV-2-LIPS-N assay did not consistently differentiate between SARS-CoV-2 positive and negative sera (Figure 7).

This study demonstrates the suitability of the SARS-CoV-2-LIPS-S assay for the sero-surveillance of SARS-CoV-2 infection in a range of animal species. The findings were published in the journal *Viruses* (Berguido et al (2021). DOI:10.3390/v13081649).

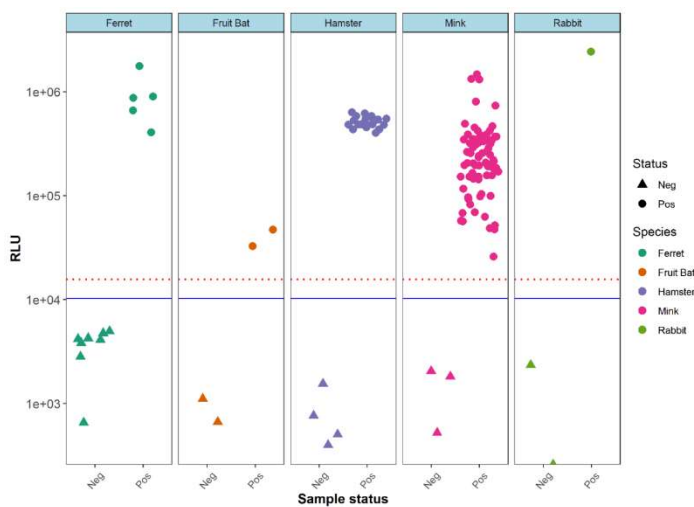


Figure 7: Distribution of the LIPS-S assay antibody values based on the sample's known SARS-CoV-2 antibody neutralization status. Note, that all negative samples are located below the blue threshold line of mean plus 3 standard deviations and all positive samples are located above it.

Comparison of Eight Diagnostic in Vitro Assays for the Detection of African Swine Fever Virus

With the recent spread of the African swine fever (ASF) in Europe, Asia, and the Caribbean region, after being endemic for decades in Africa, PCR-based commercial kits and various master mixes are increasingly being used in addition to the OIE recommended protocol from King et al. (2003) (World Organisation for Animal Health, 2021). Often, the availability and cost of commercial kits or master mixes can be a limiting factor for diagnostic laboratories, in addition to the requirements for transportation and storage of temperature-sensitive reagents in remote areas. In such cases, alternatives should be ready to maximize surveillance and mining of ASF.

To evaluate alternatives, the APH Laboratory tested five commercial quantitative real-time PCR (qPCR) master mixes from Thermo Fisher Scientific, Bio-Rad, Biotechrabbit, Promega and Qiagen using the same primers and probe mix derived from the King et al. (2003) protocol.

The sensitivity, specificity, correlation and inter-assay agreement were assessed. We further included three ad hoc molecular diagnostic kits VetMax™ African Swine Fever Virus Detection Kit (ThermoFisher), ID Gene African Swine Fever Duplex (ID-Vet) and Virotype ASF PCR Kit (Qiagen/Indical). The limit of detection (LOD) was assessed for each assay. The comparative study panel comprised 83 archived DNA samples from ASFV clinical samples, belonging to five different genotypes from outbreaks in 16 countries in Asia and Africa. The analytical specificity was assessed against a panel of swine pathogens. The LOD ranged from 13 to 41 gene copies per reaction. VetMax exhibited the lowest detection limit (13 gene copies per reaction) and Bio-Rad the highest detection limit (41 gene copies per reaction). Cq values obtained from the lowest dilution, in which all replicates ($n = 25$) could still be amplified (50 gene copies per reaction), were not significantly different between kits, according to the Kruskal-Wallis test. Inter-assay agreement was assessed using the Fleiss-Kappa statistical test and was shown to be excellent in all cases. Agreement using statistical test Bland-Altman was good for samples with Cq values < 25 and moderate for Cq values > 25 (Figure 8). We conclude that all the assays evaluated in this study can be used for the routine detection of ASFV.

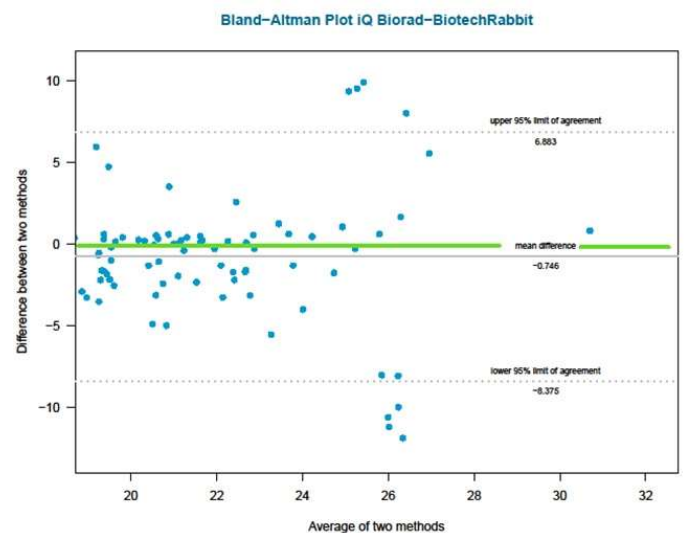


Figure 8: The Bland Altman plot shows differences between the Cq values of the comparator (Bio-Rad) and the tested assay (Biotechrabbit) against the average of the Cq values. On average, Biotechrabbit had 0.746 lower Cq-values as compared to Bio-Rad. The green lines represent the scenario of identity with perfect agreement. The grey lines represent the bias between the test assays and the comparator. The grey dotted lines represent the limits (upper and lower) of agreements.

Porcine Circovirus-2 in Africa: Identification of Continent-Specific Clusters and Evidence of Independent Viral Introductions from Europe, North America and Asia

Porcine circovirus-2 (PCV-2) is associated with several disease syndromes in domestic pigs that have a significant impact on global pig production and health. Currently, little is known about the status of PCV-2 in Africa.

A total of 408 archived DNA samples collected from pigs in Burkina Faso, Cameroon, Cape Verde, Ethiopia, the Democratic Republic of the Congo, Mozambique, Nigeria, Senegal, Tanzania, and Zambia between 2000 and 2018 were screened by PCR for the presence of PCV-2. Positive amplicons of the gene encoding the viral capsid protein (ORF2) were sequenced to determine the genotypes circulating in each country.

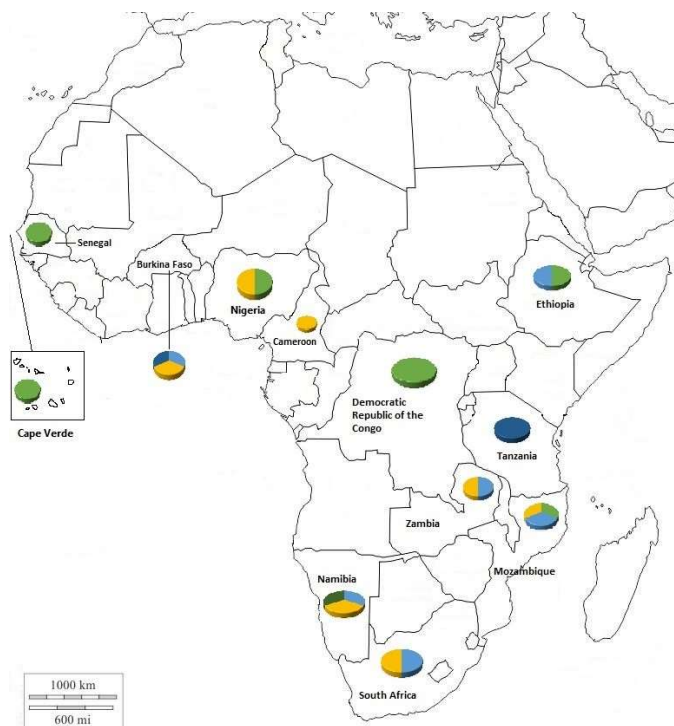


Figure 9: Distribution of PCV-2 genotypes in Africa. Colours in the circles indicate the viral genotypes identified in each country: light green: PCV-2a; light blue: PCV-2b; yellow: PCV-2d; dark green: PCV-2c; dark blue: PCV-2g. Although not discussed in detail in the present study, the presence of PCV-2c in warthogs in Namibia is also indicated

Four of the nine currently known genotypes of PCV-2 were identified (i.e. PCV-2a, PCV-2b, PCV-2d and PCV-2g) with more than one genotype being identified in Burkina Faso, Ethiopia, Nigeria, Mozambique, Senegal and Zambia (Figure 9). Additionally, a phylogeographic analysis which included 38 additional ORF2 gene sequences of PCV-2s previously identified in Mozambique, Namibia and South Africa from 2014 to 2016 and 2019 to 2020 and available in public databases, demonstrated the existence of several

African-specific clusters and estimated the approximate time of introduction of PCV-2s into Africa from other continents. The data generated have important implications for pig production at both the small-holder and commercial farm level on the continent.

Identification of Porcine Circovirus-3 in Mozambique

Porcine circoviruses are small ssDNA viruses that belong to the genus *Circovirus* of the family *Circoviridae*. Currently, four species of PCVs have been identified, namely PCV-1, PCV-2, PCV-3 and PCV-4. Porcine circovirus 3 (PCV-3) has been associated with an assortment of clinical conditions in pigs and has been reported in many countries worldwide. In Africa there is no data on the presence of PCV-3, which led to the following study.

An archive of 172 DNA samples consisting of spleen, tonsils, liver and ganglia were taken from 91 pigs and stored at -20°C. The samples, which originated from a study on the presence of ASF virus in Mozambique, were screened for PCV-3 by PCR targeting the capsid gene (ORF2) of the PCV-3 genome. The samples were collected between 2011 and 2019 from all provinces of Mozambique except Cabo Delgado. PCR amplicons were purified and sequenced commercially by LGC Genomics (Berlin, Germany). The sequences were edited and assembled and a neighbor-joining phylogenetic tree based on raw genetic distances was reconstructed using MEGA7.

The reliability of the sequence cluster was evaluated by performing 1000 bootstrap replicates. Seven animals (7.5%) were positive for PCV-3. Of these samples, all except one were also positive for ASF virus. Three of the samples were collected from three pigs from the same farm in August 2016, while the other samples were collected at different times from individual animals from separate districts of Maputo province.

All of the ORF2 sequences belonged to Clade 1, as defined by Franzo et al (2020). Phylogenetic analysis and comparison with other ORF2 sequences from PCV-3s available in GenBank confirmed the heterogeneity of the samples and the variation in the nucleotide sequence of the ORF2 (Figure X). The analysis also indicated an epidemiological link between the PCV-3 strains identified in Mozambique and other PCV-3s reported in North and South America, Asia, and Europe.

This is the first identification of PCV-3 in Mozambique (and Africa) and the first evidence of co-infection of PCV-3 and ASF virus. The study should provide a starting point for further investigation into the presence and impact of PCV-3 in Africa.

Evidence for Co-Infection of Pigs with African Swine Fever and Porcine Circovirus-2

Together with porcine parvoviruses and pseudorabies virus, porcine circoviruses (PCVs) and African swine fever (ASF) virus are considered the four main DNA viruses that significantly affect swine health. Porcine circovirus infections are typically associated with lymphoid depletion and immunosuppression, so infected pigs are often more susceptible to other viruses and bacteria. Many examples of co-infections of PCVs with other pathogens have been reported but no information on the co-infection of Porcine circovirus 2 (PCV-2) and/or Porcine circovirus 3 (PCV-3) and ASF virus is currently available, so this study was undertaken to determine whether such co-infections could be detected.

Archival swine DNA samples from Indonesia and Mongolia, some of which were previously shown to be positive for the ASF virus, were screened for the presence of Porcine (PCV-2 and Porcine circovirus 3 PCV-3) by PCR.

Samples from both countries were positive for PCV-2 (n=3 from Mongolia and n=2 from Indonesia) while none were positive for PCV-3. The PCV-2 amplicons were sequenced and phylogenetic analyses revealed that the PCV-2 strains belonged to four different genotypes: PCV-2a (Mongolia), PCV-2b (Mongolia and Indonesia), PCV-2d (Indonesia) and PCV-2g (Mongolia).

This is the first report of ASF virus/PCV-2 co-infection in pigs and the first report of the presence of PCV-2 in Mongolia. Whether PCV-2 infection and the associated immunosuppression predisposed the pigs to the ASF virus secondary infection is unclear due to the limited number of positive samples and lack of supporting epidemiological evidence. Nevertheless, this study should encourage further investigations with larger samples sizes to determine whether there is any correlation between PCV-2 occurrence and ASF virus infection, epidemiology, and pathogenesis.

The Oryx Antelope: an Unexpected Host for Porcine Circovirus-2 (PCV-2)

For several years after its discovery, Porcine circovirus 2 (PCV-2) represented a major threat to the swine industry through economic losses due to the associated clinical syndromes, decreased production in both symptomatic and asymptomatic animals and disease management costs. Widespread vaccination administration has largely reduced the impact of this infection and represents the most effective control measure. However, the efficacy of vaccination is threatened by the emergence of novel (or uncommon) PCV-2 genotypes.

In addition to domestic pigs, PCV-2 has been detected in several other species, a fact which could have an impact on new variant emergence and maintenance.

Considering this, the present study assessed the distribution of the minor PCV-2c genotype in non-Suidae ungulates in Namibia. Red hartebeests (*Alcelaphus buselaphus caama*) (n = 44), kudu (*Tragelaphus strepsiceros*) (n = 10) and oryxes (*Oryx gazella*) (n = 54), whose mediastinal lymph nodes were sampled after slaughtering during the period 2019–2021, were included in the study. Two oryxes (3.7%; 95% CI = 0.45–12.75%) were PCV-2-positive according to PCR. Complete genome sequences were obtained for the two samples, identifying them as PCV-2c genotypes. The sequences were identical and shared a high percentage of identity (~99.9%) with those recently obtained from warthogs living in the same area.

The present study confirms the presence of the PCV-2c genotype (previously considered extinct) in Namibian wild animal populations and demonstrates greater than expected PCV-2 host plasticity. Because of the role these niches can have in the maintenance and evolution of minor PCV-2 genotypes, more extensive and dedicated studies should be performed to prepare authorities to promptly react to potential emerging threats from these viruses.

Animal Genetics

Application of Nuclear and Genomic Tools to Enable for the Selection of Animals with Enhanced Productivity Traits (CRP D3.10.28)

- **Testing and validation of new world camelid SNP panel in the multi-species camelid microarray**

Animal Production and Health Laboratory (APHL) in collaboration with the Veterinary Medical University (Austria) and International Camel Genome Consortium, developed a multi-species camelid DNA chip for characterization and selection of high producing camels.



New world camelids of Peru

This novel multi-species chip contains ~200,000 single nucleotide polymorphic (SNP) markers, with >60,000 (60K) from each of dromedary, Bactrian and new world camelid species groups (Figure 10). To validate the new world camelid SNP, 280 samples collected from four different species (Alpaca, Llama, Vicugna and Guanaco) were analyzed on the array. The raw signals were utilized to generate genotyping library files specific for new world camelids. The validation process was successful, with extraction of genotypes at more than 53,000 marker loci and a success rate of 88.47%.

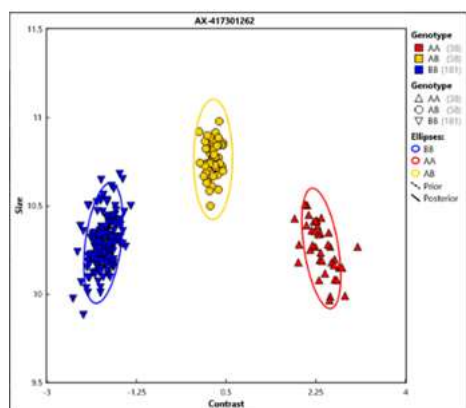


Figure 10: A sample new world camelid SNP cluster-plot from multi-species camelid microarray

Marker Metrics Summary

- Number of Markers: 59995
- Number of BestandRecommended: 53077
- Percent BestandRecommended: 88469

ConversionType	Count	Percentage
PolyHighResolution	30462	50.774
NoMinorHom	14181	23.637
MonoHighResolution	8434	14.058
Other	4410	7.351
OTV	1553	2.589
CallRateBelowThreshold	955	1.592

Table 1: Marker metric summary for new world camelid SNP panel

The thresholds for quality control parameters were set high with DQC>0.82, SNP QC call rate >97%, average call rate for passing samples ≥ 98.5 and percent passing samples ≥ 95 . About 30,400 (~50.7%) markers were classified under PolyHigh Resolution (presence of both homozygotes and heterozygotes) category, ~14100 markers (23.64%) under NoMinor Homozygotes (absence of minor allele homozygotes) category and ~8400 (~14%) under MonoHigh Resolution (monomorphic) category (Table 1). The successful validation will enable genetic and genome wide evaluation of new world camelid species. The camelid array is now ready for transfer to member states.

Further genotyping of diverse old and new world camelid populations for biodiversity studies is currently under progress.

• Whole genome radiation hybrid (RH) mapping of dromedary – Characterization and genotype extraction

Genomic resources such as whole genome linkage maps and reference genome assemblies are scarcely available for camelid species. In 2018, the APH Laboratory in Seibersdorf completed the development of two radiation hybrid (RH) panels (5000RAD and 15000RAD) for dromedary camel to establish whole genome radiation hybrid maps.

Experimental designs were formulated to characterize the 5000RAD RH panel by using the multi species camelid array developed and validated at APH Laboratory. The summarized signal intensities were extracted from raw data using the Axiom Analysis Suite. Various statistical approaches were considered for typing RH panels and the K-Means clustering approach was selected to determine the presence or absence of a given marker in the RH clones. Several statistical parameters (cluster quality, distance between centroids of clusters, variance of clusters, assignment of controls to appropriate clusters) were utilized to optimize and classify the RH clones based on raw signal intensity data.

The RH genotypes for all the SNP markers available on the array (Dromedary, Bactrian and Alpaca SNPs) were generated. The binary data (1-positive and 0-negative) for the 5000RAD RH panel were generated and the input file for CarthaGene software was generated successfully (Figure 11).

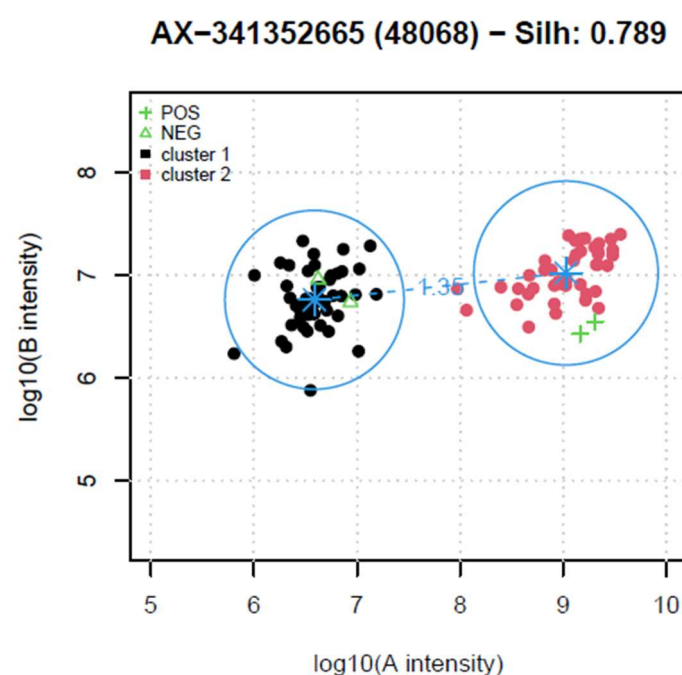


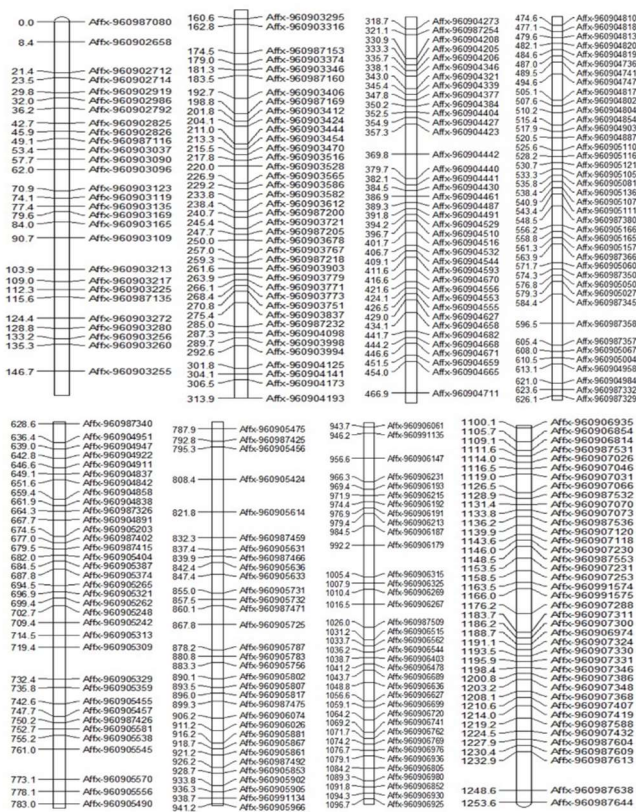
Figure 11: K-Means clustering approach to genotype RH clones using signal intensity data

• Whole genome radiation hybrid (RH) mapping of dromedary – Chromosome level mapping

As a first step towards chromosome level mapping, SNP markers from dromedary alone were considered to construct the framework map. A stringent set of criteria on clustering parameters was applied to select vectors for the analysis. Only vectors with good control values (POS_ok=TRUE, POS_N>0) and high clustering (Ksilh>6.0, and KcentreDiff>6.0) with less than 3 dubious positions (POS_out<3) were used in the final dataset. Resulting vectors were inspected visually and outliers removed manually. The markers were finally analyzed using CarthaGene software installed on a Linux platform to generate whole genome radiation hybrid maps. The markers that formed different linkage groups were compared with available genomic coordinates to establish chromosome level maps for dromedary (Figure 12).

b) to associate production performance with different levels of taurine admixture under small holder production systems.

APH Laboratory provided technical and scientific support to the project team for performing genome wide analysis and estimation of genetic admixture in crossbred cattle. A total of 1114 cattle (977 crossbreds, 79 purebred zebu and 58 purebred Holstein cattle) located in four administrative divisions of Bangladesh were genotyped using a 60K bovine SNP array. The genotype data were used to estimate the level of taurine admixture and classify the crossbred cattle into six different groups: $\geq 87.5\%$, 75.0% to $<87.5\%$, 62.5% to $<75.0\%$, 50.0% to $<62.5\%$, 25.0% to $<50.0\%$ & $<25.0\%$ of taurine blood. Significant differences were revealed in the level of taurine admixture among crossbred cattle located in different regions of Bangladesh (Figure 13). Phenotypic evaluation and comparison of performance (milk production and reproduction traits) among different crossbred genetic groups is currently under progress.



• Detection of selection signatures related to high altitude adaptation in Peruvian cattle

Cattle in Peru are managed in diverse production systems with varying climatic factors. Cattle are reared in high altitudes ranging from 1000 to 4800m above mean sea level (MSL). Over a period of many years, the local Creole cattle has been “graded up” with the Brown Swiss breed to improve milk and meat production, but still retain the traits related to high altitude adaptation. The CRP D31028 research contract on “Genomic data from dairy cattle under different climatic conditions in Peru” aimed a) to determine the level of genetic admixture in upgraded local cattle and b) to detect selection signatures related to high altitude adaptation in Peruvian cattle. APH Laboratory provided technical and scientific support to perform genomic evaluation and detection of selection signatures in Peruvian cattle.

A total of 574 cattle (322 Brown Swiss, 191 Creole and 61 Holstein cattle) located in high and low altitude regions were genotyped using a 60K bovine SNP array. The genotype data was used to estimate basic biodiversity measures, inbreeding, and genetic admixture levels in Peruvian cattle. The habitats of sampled cattle from each breed were classified as high or low altitude depending on their location. Two genome scan approaches based on extended haplotype homozygosity statistics (XP-EHH: Cross Population Extended Haplotype Homozygosity and Rsb scores) were utilized to detect signatures of selection. A total of 28 gene ontology terms (e.g., chitin metabolic process, amino sugar catabolic process, etc.) were identified to be impacted among the high and low altitude Creole genomes. Further analysis of data is currently under progress (Figure 14).

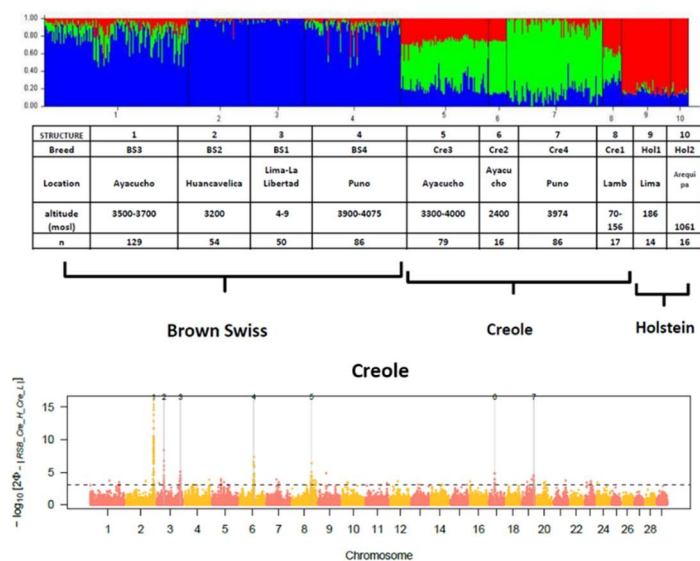


Figure 14: Genetic admixture and selection signature analysis in Peruvian cattle

• Genome-wide association study on production and reproduction traits in Serbian Holstein cattle

The CRP D31028 research contract on “Animal identification, pedigree, exterior and performance data recording in selected Holstein-Friesian cattle population in Serbia used for future genetic selection under AI program” aimed a) to record pedigree and phenotype data on Serbian Holstein cattle in select dairy farms, and b) to perform genome wide association study (GWAS) on production and reproduction traits in Serbian Holstein cattle. The APH Laboratory provided technical and scientific support to perform genotyping and GWAS.

A total of 336 cows and 20 bulls were genotyped using a 60K bovine SNP array on Affymetrix Axiom platform. The daughter design was utilized successfully to perform genome wide association studies (GWAS) on various traits including 305 days milk yield, lactation milk yield, 305 days fat yield, lactation fat yield, protein percentage, first service period, first calving interval and first dry period (Figure 15).

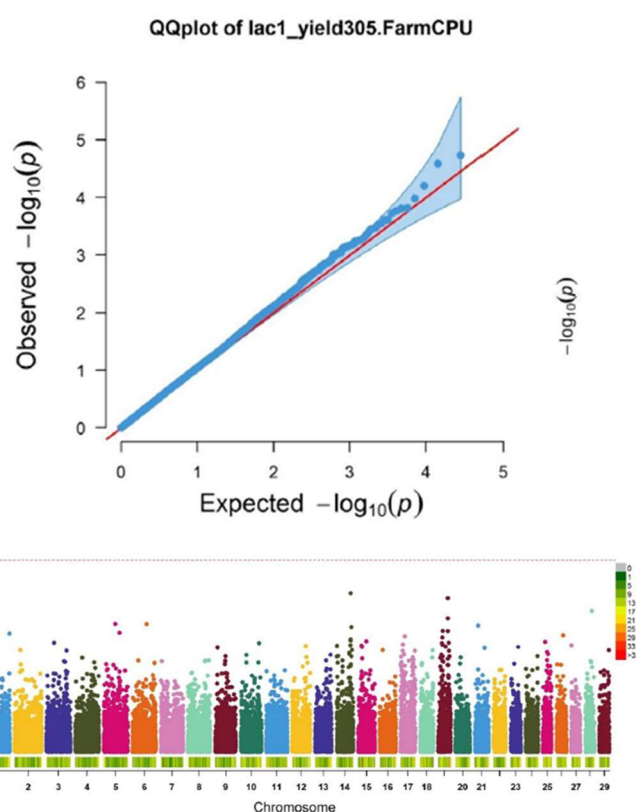


Figure 15: Genome wide association study in Serbian Holstein cattle

Implementation of the Global Plan of Action for Animal Genetic Resources

Building capacities in member states for effective management and utilization of AnGR

One of the strategic priority areas enshrined in the Global Plan of Action for Animal Genetic Resources (GPA-AnGR) is to assist countries in building capacities and enabling country-driven implementation of activities related to documentation, characterization, sustainable use and development of locally available livestock breeds/populations. The APH Laboratory continues its efforts to improve the capacity of the Member States (MSs) to implement advanced nuclear-related genomic technologies for efficient management and utilization of locally available animal genetic resources. Three virtual training courses were organized with an aim to improve the skills of geneticists/breeders in developing countries.

The special focus of the courses was on bioinformatics analysis of livestock genomic data related to biodiversity assessment, genetic evaluation, and phenotype-genotype association studies.

- **Virtual Regional Training Course on “Genetic characterization of livestock breeds - Bioinformatics analysis of multi locus genotype data”** (Read more on the page 10 of this issue)
- **Virtual National Training Course on “Phenotype recording and conventional breeding methods for Cashmere goat improvement”** (Read more on the page 10 of this issue)
- **Virtual Regional Training on “Bioinformatics data analysis for biodiversity and genome-wide association studies in livestock** (Read more on the page 14 of this issue)

Fellows, Interns and Consultants

Ms Fatima Liaqat joined the Joint FAO/IAEA Centre as an intern on 1 September 2021. Ms Liaqat is an MS-Graduate of Microbiology & Immunology. She will work within the domain of vaccine development by contributing to the work of the APH Laboratory on the development of assays to measure mucosal immunity induced by vaccines. Ms Liaqat is also responsible for assisting the investigation of irradiated vaccines for transboundary and zoonotic diseases.

Mr Hatem Ouled Ahmed Ben Ali joined the Joint FAO/IAEA Centre as a consultant on 1 September 2021. Mr Ouled will work with the APH Laboratory team in developing and validating technologies and procedures for early detection and surveillance of transboundary animal and zoonotic diseases, emphasizing next-generation sequencing technologies. He is also involved in capacity building and technology transfer to VETLAB and ZODIAC counterparts' laboratories in the Member States.

Mr Federico Verly joined the Joint FAO/IAEA Centre as a Fellow on 6 December 2021. Holding a Master's degree in International Relations,

Mr Verly will receive training to become a trainer in data management using IAEA iVetNet platform, and to learn about standards for testing, calibration, and bio-risk management of veterinary laboratories within the framework of the Technical Cooperation Project on Supporting National and Regional Capacity in Integrated Action for Control of Zoonotic Diseases (ZODIAC project).

Mr Dejan Vidanovic joined the Joint FAO/IAEA Centre as a consultant on 1 December 2021. He will work with the APH team in developing and implementing the ZODIAC project. Mr Vidanovic is a virologist and the Head of scientific department at the Veterinary Specialized institute Kraljevo, Serbia.

Ms Lina Yu joined the Joint FAO/IAEA Centre as a consultant on 15 September 2021. Ms Yu worked with the APH Laboratory team in developing the work plan on antimicrobial resistance (AMR), and further support the laboratory capacity building through VETLAB network. With a background in pharmacy, she was one of the lead authors of the FAO Action Plan on AMR 2021-2025.

Coordinated Research Projects (CRPs)

Project Number	Ongoing CRPs	Project Officers
D31028	Application of Nuclear and Genomic Tools to Enable the Selection of Animals with Enhanced Productivity Traits	V. Tsuma M. Garcia
D31029	Quantification of Intake and Diet Selection of Ruminants Grazing Heterogeneous Pasture Using Compound Specific Stable Isotopes	V. Tsuma M. Garcia
D31030	Improving Efficiency of Animal Breeding Programs Using Nuclear Related Genomic Information – Practical Applications in Developing Countries	V. Tsuma G. Viljoen
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 G. Viljoen
D32032	Early Detection of Transboundary Animal Diseases (TADs) to Facilitate Prevention and Control through a Veterinary Diagnostic Laboratory Network (VETLAB Network)	I. Naletoski C. Lamien
D32033	Irradiation of Transboundary Animal Disease (TAD) Pathogens as Vaccines and Immune Inducers	G. Viljoen V. Wijewardana
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 G. Viljoen
D32035	Improvement of Diagnostic and Vaccine Tools for Emerging and Re-emerging Animal Health Threats	G. Viljoen V. Wijewardana
D32036	Application of Advanced Molecular Characterization Technologies Through the Veterinary Diagnostic Laboratory Network (VETLAB Network)	I. Naletoski G. Viljoen
D32037	Novel Test Approaches to Determine Efficacy and Potency of Irradiated and Other Vaccines	V. Wijewardana G. Viljoen

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

Victor Tsuma and Mario Garcia Podesta

Eleven research contracts have been awarded to institutes from various developing countries to commence project activities of this new Coordinated research Project (CRP) in 2022. The CRP aims to enable use of nuclear and related genomic technologies in Member States to enhance the efficiency of national breeding programs 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.

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

Victor Tsuma and Mario Garcia Podesta

The aim of this new Coordinated Research Project (CRP) is to enable the Member States of the IAEA, particularly among the 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, it aims to a) evaluate nitrogen and energy supplementation strategies in cattle feeding to mitigate enteric and manure GHG emission, b) 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.

Ten research contracts will be awarded in the first quarter of 2022 to institutes from various developing countries which have access to animals for controlled experiments. Among eligibility criteria are:

- a) access to institutional farms, metabolic cages/digestibility markers, and basic laboratory facilities for feed analysis, and
- b) experience and facilities for diet formulation and preparation of diets using local feed resources.

Preferably, the candidates should be able to perform gas chromatography and ^{15}N analyses, and be recipients of collateral financial support from national, bilateral, or international sources.

The research contract (RC) will last for five years and individual RC holders (RCH) will receive up to €9,000 per year to cover costs of local expenses, minor equipment, feed supplementation and analysis, and GHG emission estimation. Four Research Agreements will be awarded to institutes that have expertise in specific areas of importance to the CRP.

Irradiation of Transboundary Animal Disease (TAD) Pathogens as Vaccines and Immune Inducers (D32033)

Hermann Unger and Gerrit Viljoen

This Coordinated Research Project (CRP) started in early 2017 to continue exploring the possibilities of using irradiation in the development of vaccines. The project is built on the noteworthy results of the preceding CRP on the subject, yielding especially strong outcomes on irradiated intestinal and haemo-parasites as vaccine candidates.

However, a major shortcoming of the initial CRP was the lack of proper immunological tools to define the elicited immune responses. This issue was addressed by establishing an immunology research and development at the APH Laboratory in 2015. Since then, efforts have been made to develop assays and reagents to monitor the immune responses induced by irradiated vaccines, especially in cellular immunology. This is an area that has been neglected in livestock immunology but is of immense importance.

Among the positive outputs so far it is worth to mention the work on irradiated low pathogenic avian influenza (H9N2) and Haemonchosis in goats. In relation to the former, the counterpart in Iran has showed an excellent efficacy of irradiated influenza vaccine in broiler chicken. When it comes to the latter, the Haemonchosis irradiated larval vaccine delivered nearly 100% protection in experimental settings. Encouraged by these results, further work was done on the stability of the vaccine and establishment of the storage conditions.

Currently, a large field trial is being conducted to test the efficacy of this vaccine in Sri Lanka. This involves 240 goats between the ages 0-6 months. It is expected the results of this trial will soon pave the way to commercialization.

Moreover, the work on the irradiated vaccine against Fowl Typhoid (*Salmonella enterica* serovar Gallinarum) in Ethiopia also showed some encouraging results, although protection in the challenge birds was only partial.

The project will end in June 2022 and the final update on the CRP will appear in the next issue of the newsletter.

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 and Gerrit Viljoen

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 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.

The second research coordination meeting was held virtually from 16 June 18 June 2021. The partners of the project have collected 62 feather samples from confirmed carriers of various avian influenza viruses – AIVs (all typed by molecular techniques). The feather samples will be submitted for stable isotope analysis in order to determine the origins of the carriers if AIVs soon.

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

Gerrit Viljoen and Viskam Wijewardana

Background:

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 in rural areas. 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 for ruminants 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. Additionally, the measurement of IgA is still done by a ‘research tool’ and existing general laboratory tools must be adapted to allow their measurement in standard laboratories.

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”.

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)

Gerrit Viljoen and Viskam Wijewardana

The aim of this new Coordinated Research Project (CRP) is to complement the evaluation efforts of irradiated and other novel vaccines, as well as application of innovative tools, to determine the immune response and design immunological tools for quality control and efficacy.

The overall expected outcomes are a) new in vitro procedures for vaccine efficacy testing replacing or reducing animal challenge trials based on in vitro assays ideally employing irradiated antigens, b) evaluation of immune marker mRNA qPCR and gene expression assays, c) cytokine protein assays like ELISPOTS or ELISA, and iv) cell-based quantification assays that employ flow cytometry etc.

This CRP will not support the development of technical capacities, instead it requires the inputs from the side of each participant for us to be able to understand the immune response delivered by the specific vaccine and the basic methods of their evaluation. It is expected that these new procedures will in the future help vaccine producing labs to perform better quality control of their products. They will allow a higher confidence in the results due to a more technical approach.

The research contracts, research agreements, and technical contracts will be awarded only to applicants that have an ongoing vaccine production and/or research and preferably an active tissue culture lab, among other eligibility criteria. The applications will open in January 2022.

Please submit through following link
<https://www.iaea.org/projects/crp/d32037>

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 the following URL:

<http://cra.iaea.org/cra/index.html>

Technical Cooperation Projects

Country TC Number	Description	Technical Officer(s)
Albania ALB5008	Improving and Enhancing National Capabilities for Early Detection of Vector Borne Diseases through the Application of Conventional and Molecular Methods	I. Naletoski
Angola ANG5016	Recovering the Vaccine Production Unit and Monitoring Active Animal Immunity	V. Wijewardana
Burundi BDI5002	Improving Animal Production Through Enhanced Application of Nuclear and Related Techniques	I. Naletoski V. Tsuma
Burkina Faso BKF5021	Improving Local Poultry Production Through Incorporation of Nutraceuticals in Feeds and Genetic Characterization	V. Tsuma
Bosnia and Herzegovina BOH5002	Strengthening State Infrastructure for Food and Animal Food Control and Protecting Animal Health	I. Naletoski
Botswana BOT5018	Reducing the Incidence and Impact of Transboundary Animal and Zoonotic Diseases	C. Lamien
Botswana BOT5021	Improving Reproductive and Productive Performance of Crossbred Dairy Cattle	G. Viljoen K. Periasamy
Bulgaria BUL5017	Enhancing the National Diagnostic Capabilities for Detection of Hepatitis E Virus in Pigs and Pig Products	I. Naletoski
Belize BZE5010	Strengthening National Capacities to Control Animal Diseases	G. Viljoen
Chad CHD5008	Improving Bovine Productivity Using Artificial Insemination	V. Tsuma
Chad CHD5010	Eradicating Pests in Small Ruminants Using Nuclear Technology	M. Garcia
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
People's Republic of China CPR5025	Developing Integrated Strategies to Improve Nitrogen Utilization and Production Efficiency in Dairy Cows	G. Viljoen
El Salvador ELS014	Strengthening National Capacities for the Control of Brucellosis	I. Naletoski
Eritrea ERI5010	Increasing Small Scale Dairy Production Through Improved Feeding, Cattle Management and Higher Conception Rates, Thereby Improving Rural Livelihood and Contributing to Food Security	K. Periasamy V. Tsuma
Ethiopia ETH5020	Enhancing the Livelihood of Rural Communities through Addressing Major Zoonotic and Economically Important Small Ruminant Diseases	C. Lamien
Indonesia INS5042	Improving Cattle Productivity Through Improved Feeding and Enhanced Reproduction	K. Periasamy V. Tsuma
INT5155	Sharing Knowledge on the Sterile Insect and Related Techniques for the Integrated Area-Wide Management of Insect Pests and Human Disease Vectors	I. Naletoski
INT5157	Supporting National and Regional Capacity in Integrated Action for Control of Zoonotic Diseases	I. Naletoski

Country TC Number	Description	Technical Officer(s)
Côte d'Ivoire IVC5037	Enhancing Diagnostic Capacity for HPAI H5N1 Avian Influenza, using nuclear-derived technique	I. Naletoski
Côte d'Ivoire IVC5038	Studying Small Ruminant Respiratory Diseases	C. Lamien
Cambodia KAM5003	Supporting Sustainable Livestock Production	M. Garcia
Kenya KEN5038	Using Nuclear Techniques to Evaluate and Improve the Impact of Mutated Forages on the Performance of Smallholder Dairy Cows	M. Garcia
Kyrgyzstan KIG5001	Establishing Effective Testing and Systematic Monitoring of Residues and Food Contaminants and of Transboundary Animal Diseases	I. Naletoski
Lao P.D.R. LAO5003	Using Nuclear and Molecular Techniques for Early and Rapid Diagnosis and Control of Transboundary Animal Diseases in Livestock	G. Viljoen
Lao P.D.R. LAO5004	Enhancing National Capability for Crop Production and Controlling Trans-Boundary Animal Diseases	G. Viljoen
Lao P.D.R. LAO5005	Reducing the Incidence and Impact of Transboundary Animal and Zoonotic Diseases	G. Viljoen
Lesotho LES5006	Enhancing Animal Production and the Health of Sheep and Goats in Lesotho	G. Viljoen
Lesotho LES5007	Enhancing Livestock Production and Health	G. Viljoen
Lesotho LES5010	Using Nuclear and Molecular Technology to Improve Livestock Production and Health	G. Viljoen
Madagascar MAG5020	Improving Stockbreeding Productivity Through the Application of Nuclear and Related Techniques for Reducing Rural Poverty	I. Naletoski
Madagascar MAG5024	Applying Nuclear and DNA-Based Techniques to Improve Productivity of Local Livestock	V. Tsuma
Mauritania MAU5007	Supporting Genetic Improvement of Local Cattle Breeds and Strengthening the Control of Cross-Border Diseases - Phase II	M. Garcia
Mali MLI5026	Improving the Diagnosis of Livestock Diseases	I. Naletoski
Mali MLI5027	Using Nuclear and Molecular Techniques for Early and Rapid Diagnosis, Epidemiological Surveillance and Control of Transboundary Animal Diseases	I. Naletoski
Mali MLI5029	Upgrading Capacities to Differentiate Priority Animal and Zoonotic Diseases Using Nuclear Related Molecular Techniques	I. Naletoski
Mongolia MON5023	Enhancing Livestock Production Through the Improved Diagnosis and Prevention of Transboundary Animal Diseases	G. Viljoen
Mongolia MON5025	Improving Breed Characterization of Cashmere Goats to Facilitate the Establishment of Strategic Breeding Programmes	G. Viljoen
Morocco MOR5037	Enhancing Control of Chemical Food and Feed Contaminants, Animal Disease Diagnosis and Trade in Fresh Fruits	I. Naletoski
Mozambique MOZ5008	Strengthening National Capacity for the Application of Nuclear and Related Techniques to Improve Animal Health and Production	G. Viljoen

Country TC Number	Description	Technical Officer(s)
Mozambique MOZ5009	Strengthening National Capacity to Control the Incidence and Impact of Transboundary Animal and Zoonotic Diseases	G. Viljoen
Myanmar MYA5026	Improving the Livelihoods of Smallholder Livestock Farmers by Developing Animal Feeding Strategies for Enhanced Food Security	G. Viljoen
Myanmar MYA5028	Reducing the Incidence and Impact of Transboundary Animal and Zoonotic Diseases	G. Viljoen
Namibia NAM5018	Strengthening Animal Health and Food Safety Control Systems	G. Viljoen
Nepal NEP5004	Improving Animal Productivity and Control of Transboundary Animal Diseases using Nuclear and Molecular Techniques: Phase II	I. Naletoski
Nepal NEP5005	Strengthening Capacity in Veterinary Diagnosis	I. Naletoski
Nigeria NIR5040	Controlling Parasitic and Transboundary Animal Diseases to Improve Animal Productivity in Smallholder Farms Using Nuclear and Molecular Techniques	I. Naletoski
Nigeria NIR5041	Improving Livestock Productivity through Enhanced Nutrition and Reproduction Using Nuclear and Molecular Techniques	V. Tsuma
Pakistan PAK5052	Improving Livestock Productivity Using Nuclear and Related Techniques by Exploiting Indigenous Feed Resources while Reducing Enteric Greenhouse Gas Emissions	M. Garcia
Palestine PAL5007	Upgrading Animal Feeding Laboratory in Terms of Human Capacity Building and Infrastructure	I. Naletoski
Paraguay PAR5011	Improving the Conservation of Germplasm of High Performance Livestock and Native Cattle	M. Garcia
RAF0042	Promoting the Sustainability and Networking of National Nuclear Institutions for Development	I. Naletoski
RAF0051	Supporting Specific Needs in the African Region Due to Emergencies	I. Naletoski G. Viljoen
RAF5068	Improving Livestock Productivity through Strengthened Transboundary Animal Disease Control using Nuclear Technologies to Promote Food Security (AFRA)	C. Lamien
RAF5073	Strengthening Africa's Regional Capacity for Diagnosis of Emerging or Re-emerging Zoonotic Diseases, including Ebola Virus Disease (EVD), and Establishing Early Warning Systems	I. Naletoski
RAF5082	Enhancing Veterinary Diagnostic Laboratory Biosafety and Biosecurity Capacities to Address Threats from Zoonotic and Transboundary Animal Diseases (AFRA)	I. Naletoski
RAS5069	Complementing Conventional Approaches with Nuclear Techniques towards Flood Risk Mitigation and Post-Flood Rehabilitation Efforts in Asia	I. Naletoski
RAS5078	Enhancing Food Safety Laboratory Capabilities and Establishing a Network in Asia to Control Veterinary Drug Residues and Related Chemical Contaminants	G. Viljoen
RAS5085	Using Nuclear Derived Techniques in the Early and Rapid Detection of Priority Animal and Zoonotic Diseases with Focus on Avian Influenza	I. Naletoski
RER5023	Enhancing National Capabilities for Early and Rapid Detection of Priority Vector Borne Diseases of Animals (Including Zoonoses) by Means of Molecular Diagnostic Tools	I. Naletoski

Country TC Number	Description	Technical Officer(s)
RER5025	Improving Early Detection and Rapid Response to Potential Outbreaks of Priority Animal and Zoonotic Diseases	I. Naletoski
RER9137	Enhancing National Capabilities for Response to Nuclear and Radiological Emergencies	I. Naletoski
RLA5071	Decreasing the Parasite Infestation Rate of Sheep (ARCAL CXLIV)	M. Garcia
RLA5084	Developing Human Resources and Building Capacity of Member States in the Application of Nuclear Technology to Agriculture	I. Naletoski
Senegal SEN5042	Using Nuclear and Related Techniques in Improving the Productivity of Domestic Ruminants	V. Tsuma
Seychelles SEY5008	Building Capacity for Diagnosis of Animal Diseases using Nuclear and related Techniques (Phase I)	G. Viljoen
Serbia SRB5004	Strengthening of National Reference Laboratories Capacities for Early Detection, Epidemiological Surveillance and Control of Transboundary Animal Diseases in Emergency Situations	I. Naletoski
Sri Lanka SRL5045	Establishing a National Centre for Nuclear Agriculture	C. Lamien
Sri Lanka SRL5046	Improving Livelihoods Through Dairy Cattle Production: Women Farmers' Empowerment	M. Garcia
Sri Lanka SRL5049	Supporting Control of Stomach Worm Infection in Goats	V. Wijewardana
Kingdom of Eswatini SWA5001	Reducing the Incidence and Impact of Transboundary Animal and Zoonotic Diseases	G. Viljoen
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	M. Garcia
Tajikistan TAD5006	Applying Nuclear and Molecular Techniques for Diagnosis and Control of Transboundary Animal Diseases	I. Naletoski
Togo TOG5001	Improving and Promoting Bovine Milk Production through Artificial Insemination	V. Tsuma
Togo TOG5003	Improving Livestock Production and Milk Quality Using Artificial Insemination	V. Tsuma
Tunisia TUN5030	Enhancing Feed and Food Safety by Appropriate Management of Livestock Feed Resources for Safer Products	M. Garcia
U.R. of Tanzania URT5031	Improving Indigenous Cattle Breeds through Enhanced Artificial Insemination Service Delivery in Coastal Areas	V. Tsuma
U.R. of Tanzania URT5036	Enhancing Artificial Insemination Services and Application of Radioimmunoassay Techniques to Improve Dairy Cattle Productivity	V. Tsuma
Vietnam VIE5023	Reducing the Incidence and Impact of Transboundary Animal and Zoonotic Diseases	G. Viljoen
Zimbabwe ZIM5024	Establishing an Artificial Insemination Center to Enhance the Rebuilding of the National Herd	V. Tsuma

Publications

Books and Book Chapters

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VETLAB Network

The Veterinary Diagnostic Laboratory (VETLAB) Network is a global network of national veterinary laboratories coordinated by the Animal Production and Health Section (APH) and supported through IAEA and FAO programmatic activities as well as by South Africa through the African Renaissance Fund (ARF) and by the USA and Japan Peaceful Uses Initiative (PUI). To date, the network comprises 71 laboratories in 45 African and 19 Asian countries and is now working to expand to Central and Eastern Europe, the Caribbean and Latin America. The laboratories work with each other and experts from the Joint FAO/IAEA Centre to use nuclear, nuclear-derived and other methods for monitoring, early detection, diagnosis and control of diseases. Every year the VETLAB Network organizes ring trials, training courses and one meeting of the Directors of African and Asian laboratories. Despite the restrictions and limitations related to the current pandemic situation, in 2021 the VETLAB Network was able to conduct activities and assist partner laboratories.

For example, the organization and completion of the 2020 interlaboratory comparison for PPR and the exercise in 2021 was delayed but nonetheless organized. The meeting of the Directors of the partner laboratories was conducted on-line in October 2021 with a very high and dynamic

participation. Partner laboratories in Africa and Asia have been supported for the detection, confirmation, and control of transboundary animal diseases such as avian influenza H5N1, African Swine Fever (ASF) and Lumpy Skin Disease (LSD).

In collaboration with the Enhancing Research for Africa Network (ERFAN), in June 2021 the VETLAB Network has supported the online preparatory course on organization and management of proficiency tests co-organized by the Botswana National Veterinary Laboratory (BNVL) in Gaborone and the Central Veterinary Laboratory in Harare, Zimbabwe. In November 2021, an on-line two-weeks VETLAB training course on Sequencing and Bioinformatics also took place virtually.

More information can be found in other sections of this newsletter. We hope to fully resume all VETLAB training activities as soon as possible. APH is issuing on a regular basis the VETLAB Network Bulletin in the hope of providing a forum for participating laboratories and other stakeholders to communicate and exchange knowledge/information, to showcase achievements and to share expertise within the VETLAB Network.

The latest highlights of the VETLAB Network bulletin can be found on pages 6 and 7 of this issue.

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