# International Coordination of Research on infectious Animal Diseases (ICRAD)

A European network initiative advancing animal health and welfare

2019 – 2025

**Colophon**

**Title:** International Coordination of Research on infectious Animal Diseases (ICRAD). A European network initiative advancing animal health and welfare 2019 – 2025.

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# Introduction

This book will highlight the contributions of the ICRAD calls to the ongoing efforts of the European research community to improve animal health and welfare.

ICRAD is an ERA-NET co-fund action, including 29 funding organizations from 19 countries. This action aims to bring together the scientific community and funders from the European Union to highlight and collaborate on solutions to the most important infectious animal diseases that affect EU member states.

The scope for this ERA-NET has been developed under the SCAR Collaborative Working Group on Animal Health & Welfare Research (CWG AHW), which seeks to build further on two previous successful ERA-NETS (EMIDA & ANIHWA).

During its lifetime, ICRAD has supported 3 calls for transnational research projects within the field of Animal Health and Welfare. Through these 3 calls, ICRAD has channeled a total of € 37 million into cross border collaborative research in Animal Health and Welfare.

The 33 research projects are separated into 3 distinct sections, corresponding to the 3 ICRAD calls.

The project descriptions will provide an overview of goals and achievements, as well as visions of future activities.

We hope that this book will serve as an inspiration and reference book to researchers, funders, and other stakeholders.

Lyngby, Denmark, August 2025

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# CONTENTS

**CALL 1:**

# First international call on infectious animal diseases within ICRAD

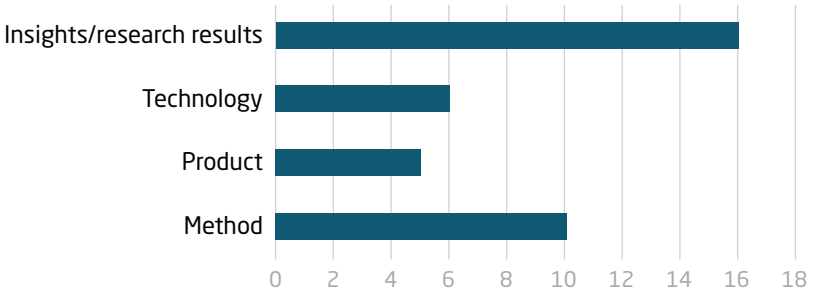
Research and innovation funded through ICRAD should seek a concerted approach towards the development of novel and improved instruments to address and control infectious diseases, particularly regarding novel detection, intervention and prevention strategies.

# Tangible outcomes of the first ICRAD Call

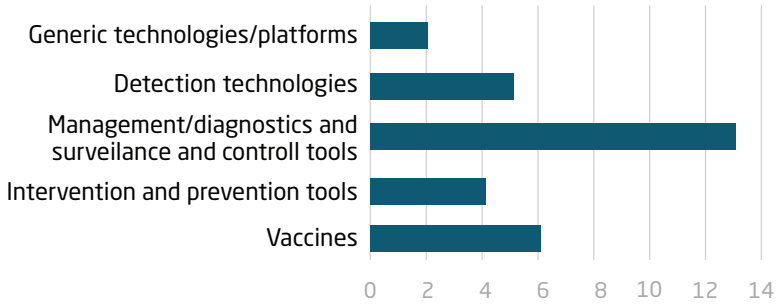
The following graphic illustrations present the tangible outcome from the first ICRAD Call. The second and third call have not been included in the summary analysis since they, at the time of this publication, are only midway and at the beginning of their project period. These additional calls are thus expected to contribute with even further tangible outcomes from ICRAD in the years to come. Also, additional outcomes from the first call may still be added to the ones already achieved.

*Acknowledgements to DEFRA, UK and PTJ, Jülich, Germany.*

## New innovations emerging from projects




## Products developed within the project lifespan or after the projet ends




*2 products are currently ready for the market

# Preventer

## Deciphering the role of influenza D virus in bovine and human respiratory diseases in Europe

**COORDINATOR:** Mariette Ducatez, INRAE, France
**PARTNERS:** Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna, Italy | University of Liege, Belgium | SLU, Sweden | Istanbul University-Cerrahpasa, Veterinary Faculty, Turkey
**PROJECT WEBSITE:** https://preventer.envt.fr
**PROJECT PERIOD:** April 2021 – January 2025

### HIGHLIGHTS/MAJOR ACHIEVEMENTS

In order to understand the role of IDV in cattle respiratory disease complex and its potential zoonotic risk, we first surveyed IDV occurrence and prevalence in the 2 species. While IDV circulation is clear in all the partners countries involved in WP1 thanks to serological data generated, IDV was detected but with low virological prevalence in European cattle. The most common a well as the most commonly detected co-infecting pathogens were P multocida and BCoV. We also evidenced traces of anti-IDV antibodies in cattle-exposed Humans.

We then aimed at specifying the synergistic and antagonistic effects between IDV and M bovis maily upon co-infections *in vitro, ex vivo* and *in vivo*. IDV co-infection tended to increase the replication of *M. bovis* with upregulation of IFN-γ. Co-localization of IDV and *M. bovis* was evidenced ex vivo in pneumocytes and bronchial cells. IDV induced expression of neutrophil-associated proteins. Whereas *M. bovis* induced expression of proteins involved in fibrin formation, IDV co-infection counteracted this expression and downregulated other acute-phase response proteins. Increased abundance of oxylipids was noted in co-infected calves.

A quantitative risk assessment modelling was finally performed to assess the zoonotic potential of IDV thanks to an expert elicitation. The effect of 4 medical (vaccination) and/or sanitary (biosecurity) mitigation measures were evaluated. An innovative tool (digital application) for assessing the level of protection of a herd using biosecurity measures against the introduction (/spread) of IDV in a farm.

### PERSPECTIVES AND RECOMMENDATIONS FOR FUTURE ACTIVITIES

The PREVENTER project will be pursued with combining surveillance data in the 5 countries, cattle and Human to understand similarities and differences between production systems and geographical areas. Dissemination activities will also be pursued.

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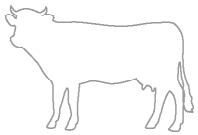
# Biosense4PrecisionMastitis



## Channel-based biosensors to support a precision agriculture approach for improved bovine mastitis management

**COORDINATOR:** Prof. Beatriz Prieto Simón, University Rovira i Virgili, Spain
**PARTNERS:** University Rovira i Virgili, Spain | Research and Innovation Centre Pro-Akademia, Poland | Seqomics Biotechnology Ltd., Hungary | Riga Stradins University, Latvia
**PROJECT WEBSITE:** beatrizprietosimon.com/biosensingbovinemastitis
**PROJECT PERIOD:** April 2021 – January 2025

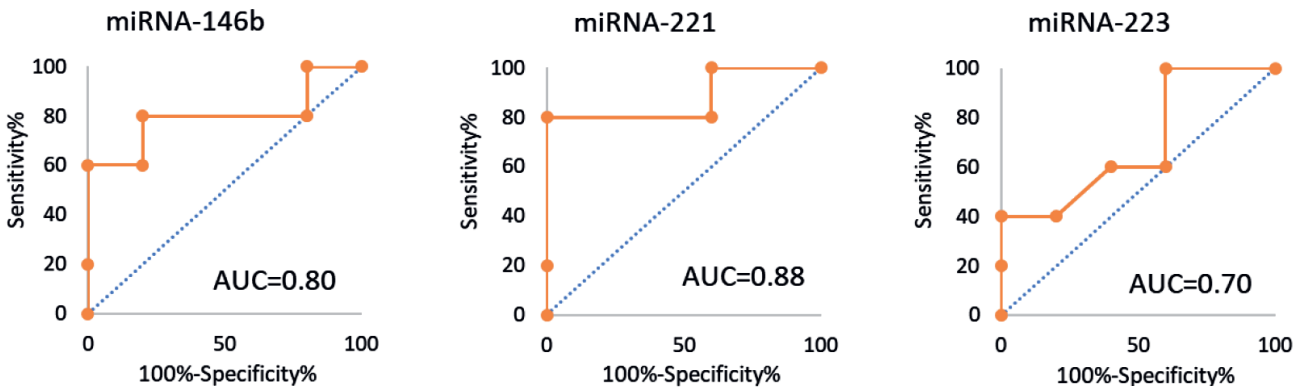
### HIGHLIGHTS/MAJOR ACHIEVEMENTS

Biosens4PrecisionMastitis has significantly contributed to advance modern diagnostics for the dairy industry, through new sensing technology for the early diagnosis of bovine mastitis. The project identified a panel of host response-derived biomarkers key during incubation (miRNA-146b, miRNA-221, miRNA-223, IL-10, β-defensin 3) as prospective mastitis biomarkers. Leveraging the presence of these biomarkers in milk, an array of electrochemical biosensors was developed for the non-invasive, stress-free and prompt diagnosis of bovine mastitis. Two sensing devices demonstrated their ability to quickly discriminate milk from healthy cows and that from cows with subclinical mastitis. The first one, harnessing the advantages of a confined nanoenvironment for the sensing event, offered high sensitivity and selectivity, enabling direct analysis of diluted milk. The second one, based on an array of electrochemical DNA (E-DNA) sensors built on gold electrodes showed outstanding diagnostic performance using milk RNA extracts. Both sensing platforms were validated in the lab with milk samples collected on farm and analysed in parallel using somatic cell count, next-generation sequencing and microbiological tests.

### PERSPECTIVES AND RECOMMENDATIONS FOR FUTURE ACTIVITIES

The technology developed represents a paradigm shift in veterinary diagnostics and anticipates timely interventions that can dramatically reduce infection severity and spread, thereby improving animal welfare and herd health. To fully exploit the potential of the project outcomes and support farmers' proactive animals' disease management, these follow-up activities are recommended:

- Direct on-farm validation of the array of E-DNA sensors;
- Standardized operating procedures and troubleshooting guidelines set to ensure the technology is practical and accessible for routine on-farm use;
- Engagement with relevant EU bodies in charge of providing scientific advice for policy making to ensure the technology meets the guidelines, standards and legislation related to animal health and welfare, and thus to streamline technology transition to support fair, healthy and resilient animal production systems;
- Technology transfer & commercialisation strategy defined to lay the foundation for business development.




ROC curves confirming miRNA-221 and miRNA-146b as reliable diagnostic biomarkers for early-stage identification of subclinical bovine mastitis cases.

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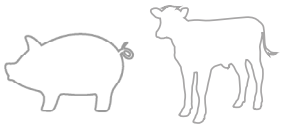
# Neovacc



## Novel strategies to enhance vaccine immunity in neonatal livestock

**COORDINATOR:** Prof Simon P. Graham, The Pirbright Institute, United Kingdom
**PARTNERS:** INRAE, France | SLU, Sweden | EPFL, Switzerland | Anses, France | OUH, Norway
**PROJECT WEBSITE:** https://www.pirbright.ac.uk/our-science/research-projects/novel-strategies-enhance-vaccine-immunity-neonatal-livestock-neovacc
**PROJECT PERIOD:** March 2021 – September 2024

### HIGHLIGHTS/MAJOR ACHIEVEMENTS

NEOVACC designed and tested three novel vaccine strategies hypothesised to enhance immune responses in neonatal animals with maternally derived antibodies (MDA):

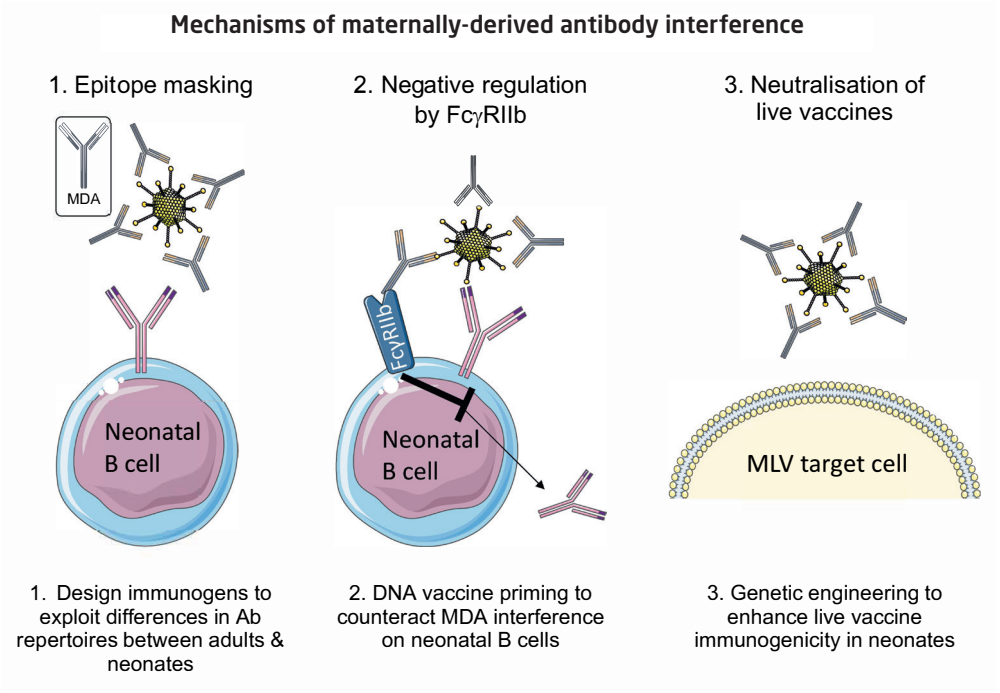
- Scaffolded epitope mimics (mimotopes) representing antigenic sites on the bovine respiratory syncytial virus prefusion F protein (BRSV preF) were designed. The antigenicity of mimotopes representing antigenic sites 2, 4 and 5 was confirmed using preF immunised calf sera. Compared to preF protein, mimotopes induced low virus neutralising antibody titres but higher titres of site 2-specific antibodies. To better understand preF epitopes targeted by MDA, monoclonal antibodies were isolated from BRSV infected adult cattle for epitope mapping studies.

- DNA-based vaccines encoding porcine reproductive and respiratory syndrome virus (PRRSV) antigens fused to moieties that target antigen-presenting cells (APCs) were assessed for their ability to prime immune responses in MDA+ piglets and augment a subsequent modified live vaccine (MLV) boost. After the MLV boost, immune responses were greater in DNA primed animals than those that received the MLV alone. The untargeted DNA prime-MLV boost group was better protected compared to the single MLV immunisation, but APC-targeting did not improve protection.

- PRRSV MLV were engineered to express peptide-based immune checkpoint inhibitors (ICIs) and their potency assessed in piglets. Immunisation with MLV expressing a dual PD-1/CTLA-4 antagonist peptide showed a trend towards improved immune responses and significantly reduced viral loads in the lungs post-challenge.

### PERSPECTIVES AND RECOMMENDATIONS FOR FUTURE ACTIVITIES

Progress has been made towards our long-term aim of developing BRSV and PRRSV vaccines capable of providing enhanced protection of neonatal cattle and piglets with MDA. We have produced novel BRSV immunogens, antigens and antibody reagents which can be used to further dissect differences in antibody repertoires between adult and neonatal calves and guide vaccine design. DNA priming was shown to enhance the subsequent response to PRRSV MLV immunisation and future studies should focus on improving the strength of this immune priming e.g., by formulation of DNA vaccines in lipid nanoparticles. The promising results obtained with the ICI-expressing MLV provides a sound basis for future research on optimizing peptide ICI-based adjuvant approaches for better control of PRRSV and other swine pathogens.




NEOVACC vaccine strategies to overcome MDA interference.

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# Rodentgate

## Future rodent management for pig and poultry health

**COORDINATOR:** Prof Dr Herwig Leirs, Department of Biology, University of Antwerpen, Belgium
**PARTNERS:** University of Greenwich, United Kingdom | University of Antwerp, Belgium | Dutch Pest & Wildlife Expertise Centre, Belgium | Julius Kühn-institute, Germany | Federal Research Centre for Cultivated Plants, NVRI, Poland
**PROJECT WEBSITE:** rodentgate.eu
**PROJECT PERIOD:** April 2021 – September 2024

### HIGHLIGHTS/MAJOR ACHIEVEMENTS

The RODENTGATE project achieved substantial advances in understanding the role of rodents in transmitting livestock pathogens in European pig and poultry farms under conditions of reduced rodenticide use. Through coordinated field sampling across five countries, over 675 rodents were trapped and screened using molecular and metagenomic tools. Pathogens of concern—including Lawsonia intracellularis, Brachyspira hyodysenteriae, Leptospira spp., and Salmonella spp.—were detected. Whole genome sequencing revealed that monophasic *Salmonella Typhimurium* strains were shared between pigs and rodents, confirming their role as active reservoirs within farm environments.

Besides describing pathogen diversity, the project made significant progress in understanding rodent-borne disease transmission dynamics by developing an innovative, empirically grounded epidemiological model. This model incorporated rodent population ecology, immunity duration, and transmission routes to simulate disease spread under different control strategies. Complementing this, the project was the first to deploy Bluetooth Low Energy (BLE) loggers to track rodent movement and social interactions, identifying key hotspots for pathogen transmission. Together, the modelling and behavioural insights showed that sanitation-based strategies—such as limiting rodent food access—are more effective and sustainable than traditional culling with rodenticides. This represents a major achievement, establishing a science-based framework for ecologically based rodent management (EBRM) tailored to modern livestock production. By combining field ecology, diagnostics, modelling, and stakeholder engagement, RODENTGATE has delivered actionable strategies for long-term, sustainable rodent management across European pig and poultry farms.

### PERSPECTIVES AND RECOMMENDATIONS FOR FUTURE ACTIVITIES

Future work should focus on scaling up ecologically based rodent control strategies and integrating real-time pathogen monitoring into farm management. Given the effectiveness of sanitation in reducing disease risks, both policy and on-farm interventions should prioritise habitat modification and resource limitation. Further metagenomic and longitudinal studies are needed to clarify transmission pathways and the persistence of antimicrobial resistance. Expanding digital tools—such as Bluetooth contact loggers and mobile platforms can strengthen surveillance and support rapid response. Continued cross-border collaboration, including farmer training and harmonised policies, will be essential to embed sustainable practices across the EU livestock sector and reduce rodenticide dependence while safeguarding public and animal health.

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# ASFVint

## Decoding a virus Achilles heel: the African swine fever virus interactome

**COORDINATOR:** Marie-Frédérique Le Potier
**DEPUTY COORDINATOR AND CONTACT:** Christopher Netherton, The Pirbright Institute, United Kingdom
**PARTNERS:** Agence Nationale de Securite Sanitaire de l'Alimentation de l'Environnement et du Travail (ANSES; Leaders), France | Friedrich-Loeffler-Institut (FLI), Germany | Institut National de Recherche pour l'Agriculture l'Alimentation et l'Environnement (INRAE), France | Instituto Nacional de Investigacion y Tecnologia Agraria y Alimentaria (INIA), Spain | The Pirbright Institute, United Kingdom | Institute of Computer Science of the University of Tartu, Estonia
**PROJECT WEBSITE:** https://www.pirbright.ac.uk/our-science/research-projects african-swine-fever-virus-interactome-asfvint
P**ROJECT PERIOD**: March 2021 – September 2024

### HIGHLIGHTS/MAJOR ACHIEVEMENTS

African swine fever virus causes a lethal haemorrhagic disease in domestic pigs and wild boar that has killed millions of animals around the world since 2007. The ASFVint project was designed to generate the first comprehensive map of how individual African swine fever virus proteins interact with cellular proteins when it infects its host. Prior to writing the project there was very little in the literature and all that was available was focused on the interactions of single viral proteins. African swine fever virus is a complicated pathogen and we aimed to identify the interactions of a large subset of the 150 or more proteins that the virus encodes. The ultimate objective was to integrate the interactions of many viral proteins to form a detailed map to identify cellular processes critical for viral replication.

We generated the first comprehensive interaction map of African swine fever virus and identified a number of important interactions. We have provided a treasure chest of candidate interactions for our groups and others to explore in the future.

We identified an interaction between viral proteins involved in the entry of virus into cells with a host protein. This in turn led to experiments that showed that drugs that interfered with this novel interaction were capable of blocking viral replication and are now being explored for use in therapeutic applications.

### PERSPECTIVES AND RECOMMENDATIONS FOR FUTURE ACTIVITIES

The complementary expertise in the project was vital for the success of the project, and generated a healthy, productive collaboration between the partners. Integration of different expertise will be of huge importance for any future project that studies protein-protein interactions. ASFVint identified the interactions of 90 viral genes, however the virus encodes for at least another 70 genes. Follow-on projects could identify the interactors of the remaining genes and complete the interactome map. African swine fever virus does not cause disease in warthogs and so exploring the interactions between virus and proteins from this host could lead to a deeper understanding of species-specific outcomes after infection with African swine fever virus.

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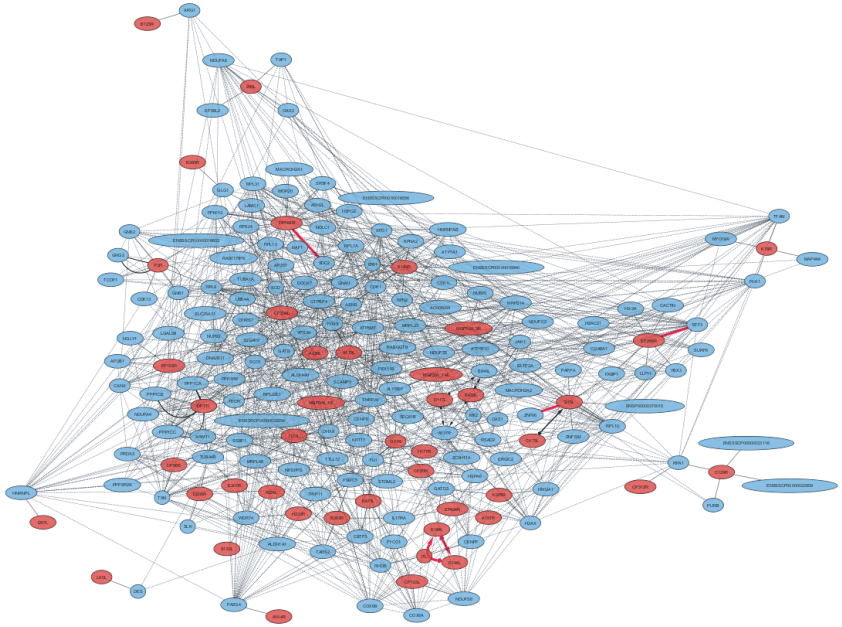

African swine fever interaction map: Blue and red boxes show host and viral proteins respectively and black lines indicate connections.


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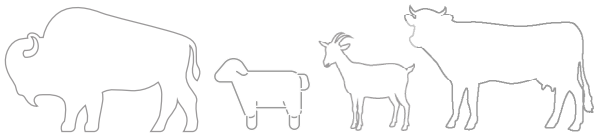
# Bruce-Geno-Prot



## A comprehensive proteogenomic analysis of Brucella to understand the epidemiology, biology, virulence mechanisms, and host-pathogen interaction

**COORDINATOR:** PD Dr. Gamal Wareth, The Institute of Bacterial Infections and Zoonoses (IBIZ), Fredrich-Loeffler-Institut (FLI), 07743 Jena, Germany

**PARTNERS:** FLI, Germany | Pendik Veterinary Control Institute, Turkey | Harran University, Turkey | Crete University, Greece

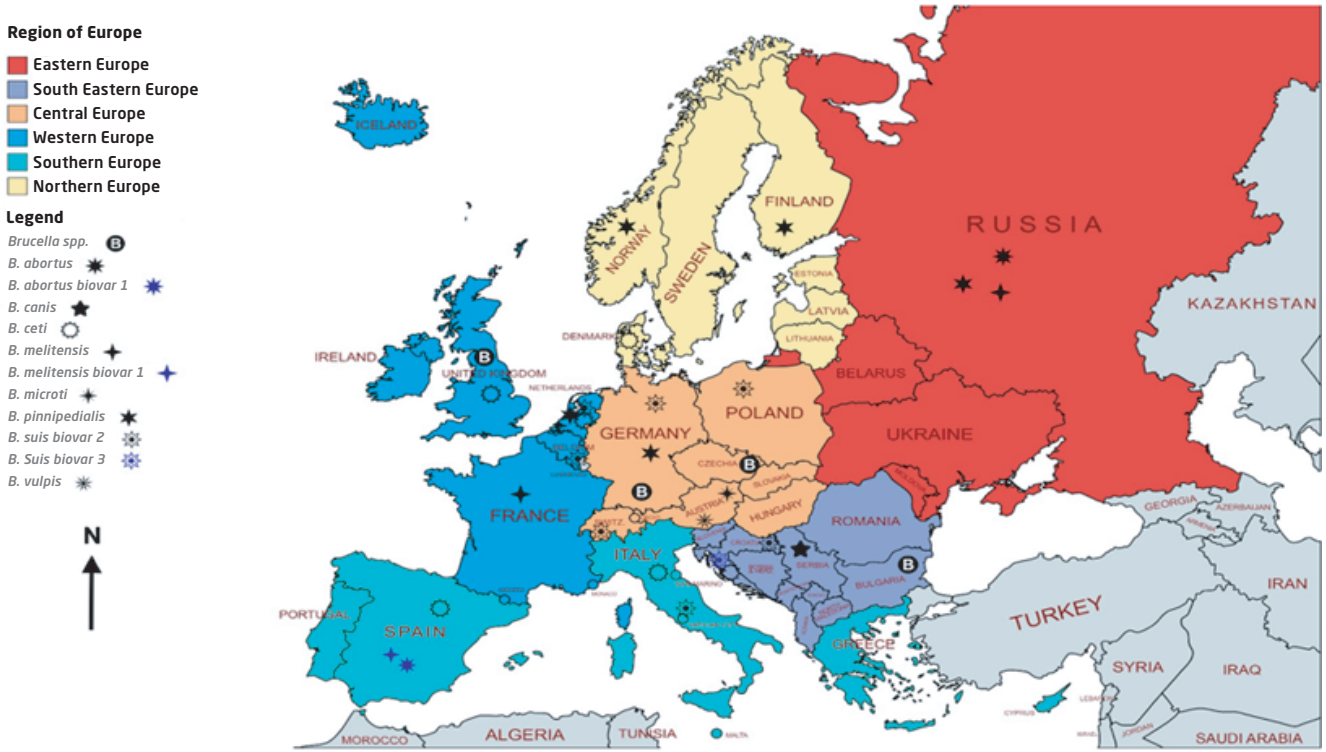
**PROJECT PERIOD**: April 2021 – September 2024

### HIGHLIGHTS/MAJOR ACHIEVEMENTS

Brucellosis in wildlife across European countries has been thoroughly evaluated, clarifying the role of wildlife in the maintenance and spread of the disease, as well as the geographical and host distribution of brucellosis in various wildlife species. Next-generation sequencing (NGS) and proteomic technologies have been employed to analyze a large number of *Brucella* isolates, which has led to a deeper understanding of the genome and proteome of Brucella. A comprehensive assessment has revealed genetic and protein differences between *B. abortus* and *B. melitensis*, which were isolated from various hosts, including cattle, buffalo, sheep, goats, and humans. This research has identified genes and proteins associated with virulence and pathogenicity. Additionally, studies have examined the differences in adhesion and invasion between *B. abortus* and *B. melitensis* in bovine and ovine cell lines, as well as the stability of some genes under stress conditions. The project has standardized also the methodology and advanced protocols for the genomic and proteomic analysis of *Brucella* species.

### PERSPECTIVES AND RECOMMENDATIONS FOR FUTURE ACTIVITIES

The project aimed to identify and monitor the presence of *Brucella* in the environment and wildlife ecosystems, which is crucial for public health and community settings. Understanding the role of these reservoirs in the epidemiology and transmission of brucellosis is essential. Furthermore, a comprehensive understanding of the genomic and proteomic contents of *Brucella* will contribute to a better understanding of its biology, improve the development of species-specific treatment in humans, design better diagnostic tools and vaccines, and clarify several aspects of *Brucella* pathogenesis. The data obtained from whole genome sequencing (WGS), proteomic analyses, cell culture experiments, and culturing of *Brucella* under stress will contribute to unraveling the mystery of host specificity and host-pathogen interaction. This knowledge will help in understanding the mechanisms of infection and in developing strategies to prevent the spread of the disease.




Distribution of Brucella species and biovars in European countries in wildlife species.

## PUBLICATIONS IN PEER REVIEWED INTERNATIONAL JOURNALS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# FluNuance

## Virulent Non-Notifiable Avian Influenza; Determinants of virulence of emerging viruses

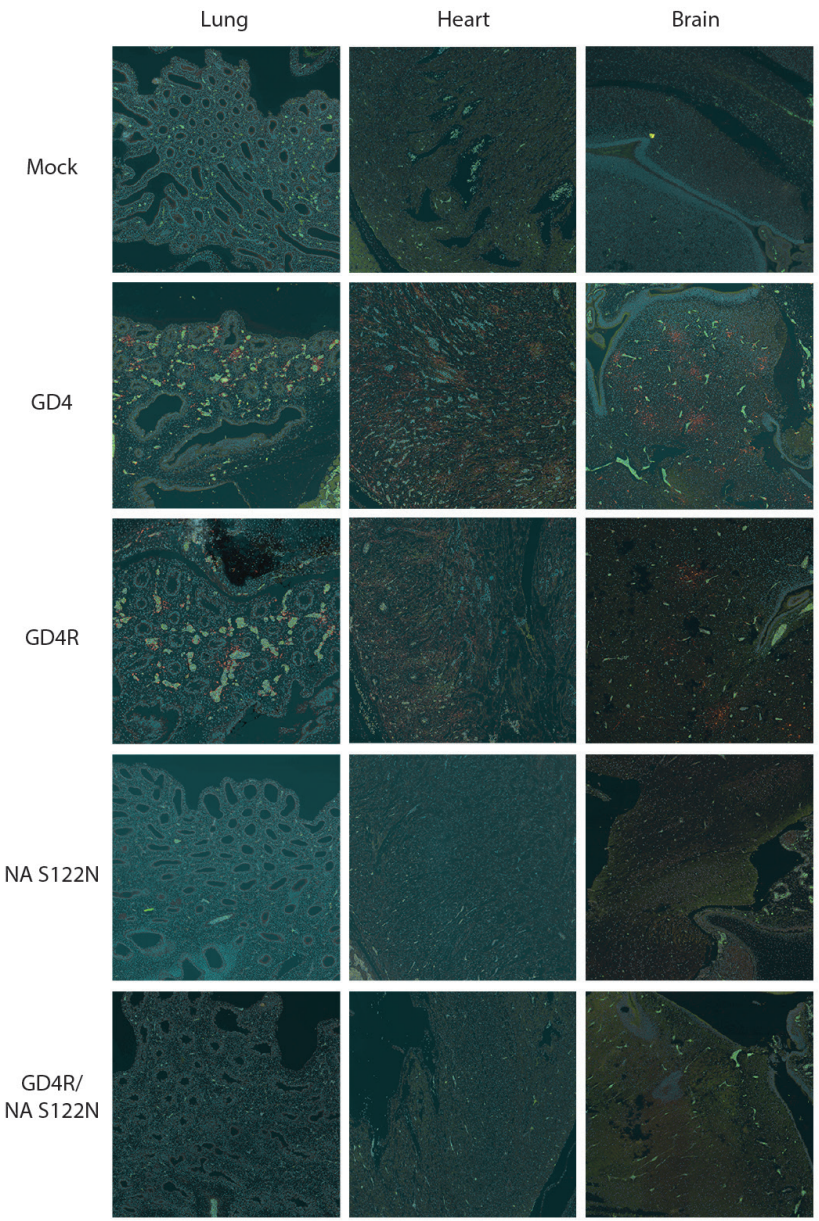
**COORDINATOR**: Prof. Dr J.J. de Wit, University of Utrecht, The Netherlands
**PARTNERS**: Royal GD, The Netherlands | University of Edinburgh, United Kingdom | Roslin Institute Infection and Immunity, United Kingdom | Stiftung Tierartzliche Hochschule Hannover Clinic for Poultry, Germany | PIWET Department of Poultry Diseases, Poland | National Food Chain Safety Office Veterinary Diagnostic Directorate Virology, Hungary
**PROJECT PERIOD:** March 2021 – December 2024

### HIGHLIGHTS/MAJOR ACHIEVEMENTS

- Molecular determinants of the virus contribute to the virulence of the LPAIV, which go beyond the characteristics of the hemagglutinin (HA) normally investigated for classification of AIV into HPAI and LPAI pathotypes.
- Presence of mutations in the neuraminidase (NA) gene that enable plasminogen binding to activate HA could be an important virulence factor.
- NA-mediated plasminogen recruitment and unusual HA cleavage site work synergistically to increase virus spread *in vitro* and *ex vivo.*
- Infection studies in embryonated eggs and organ cultures provide suitable tools to supplement the molecular characterization of newly emerging LPAIV, and subsequently allow a possible risk assessment.
- The more virulent the virus the higher the virus titres or more systemic spread in the embryo was found.

### PERSPECTIVES AND RECOMMENDATIONS FOR FUTURE ACTIVITIES

Our work will help national surveillance programs involved in the monitoring of avian influenza by better assessing the level of pathogenicity of LPAI viruses. The tools validated in this project to support (inter)national surveillance are ready for the market. Improved surveillance and decision making regarding LPAI infections will ultimately lower the impact of LPAI outbreaks in poultry and thereby lowering the risk for zoonotic infections affecting human health.




Immunofluorescence staining of viral nucleoprotein (NP) in the lung, heart and brain of chicken embryos infected with the indicated viruses. Red - NP, blue - nucleus, green - autofluorescence.

# TechPEPCon

## Use of frontline technologies to screen pathogens, environment and pigs for a better disease control in swine herds

**COORDINATOR:** Hans Nauwynck, Laboratory of Virology, Department of Translational Physiology, Infectiology and Public Health, Ghent University, Belgium
**PARTNERS:** IZSLER, Italy | UW, Poland | AUTh, Greece | UVMB, Hungary
**PROJECT WEBSITE**: https://techpepcon.ugent.be
**PROJECT PERIOD**: March 2021 – November 2024

### HIGHLIGHTS/MAJOR ACHIEVEMENTS

With TechPEPCon, new digital frontline technologies (third generation nanopore sequencing (PathoSense), digital biosecurity analysis (Biocheck) and climate sensing (Healthy Climate Solutions) were implemented to analyze (i) infections that are occurring on healthy and clinically affected farms and may cause disease, (ii) biosecurity and (iii) stable climate. On 'healthy' farms, it was demonstrated that (i) a large number of respiratory, intestinal and general viruses and bacteria are enzootically circulating, mainly causing subclinical infections; (ii) periods of clinical signs were present and accepted by the farmer as being normal and (iii) biosecurity and stable climate could be improved. On 'affected' farms, the combined use of these digital frontline technologies allowed to make correct diagnoses, which would be much more difficult with the current way of making diagnoses (detection of selected pathogens). It became clear that the value of the 'diagnosis of one pathogen' is restricted. Pigs are continuous under an 'immunostimulation' status, which is negative for their growth and allows viruses with a tropism for lymphoblasts (parvo- and circoviruses) to damage the immune system and to evolve fast. The digital diagnostic platform allows to follow up viruses and bacteria in real time and to identify emerging pathogens.

### PERSPECTIVES AND RECOMMENDATIONS FOR FUTURE ACTIVITIES

- At present, the visual and auditive digital data that are collected in swine herds are of little use for veterinarians. More efforts should be made to develop usable software for swine veterinarians.
- It is recommended to centralize the digital data from third generation diagnostic platforms at a national and European level and open it for practitioners and decision-making authorities.
- Veterinary students and veterinarians should be trained to use the new digital diagnostic tools in a proper way.
- The number of infections that pigs experience in their life should be reduced to improve their growth and health/welfare and to reduce the evolution of circo- and parvoviruses.

## PUBLICATIONS IN PEER REVIEWED INTERNATIONAL JOURNALS

Vereecke, N., Wozniak, A., Pauwels, M., Coppens, S., Nauwynck, H., Cybulski, P., Theuns, S., 2023. Successful whole genome nanopore sequencing of swine influenza A virus (swIAV) directly from oral fluids collected in Polish pig herds. Viruses, 15, 435

# IFNASF

## Characterization of virus- and host-specific modulation of type I IFN in African swine fever virus virulence or attenuation

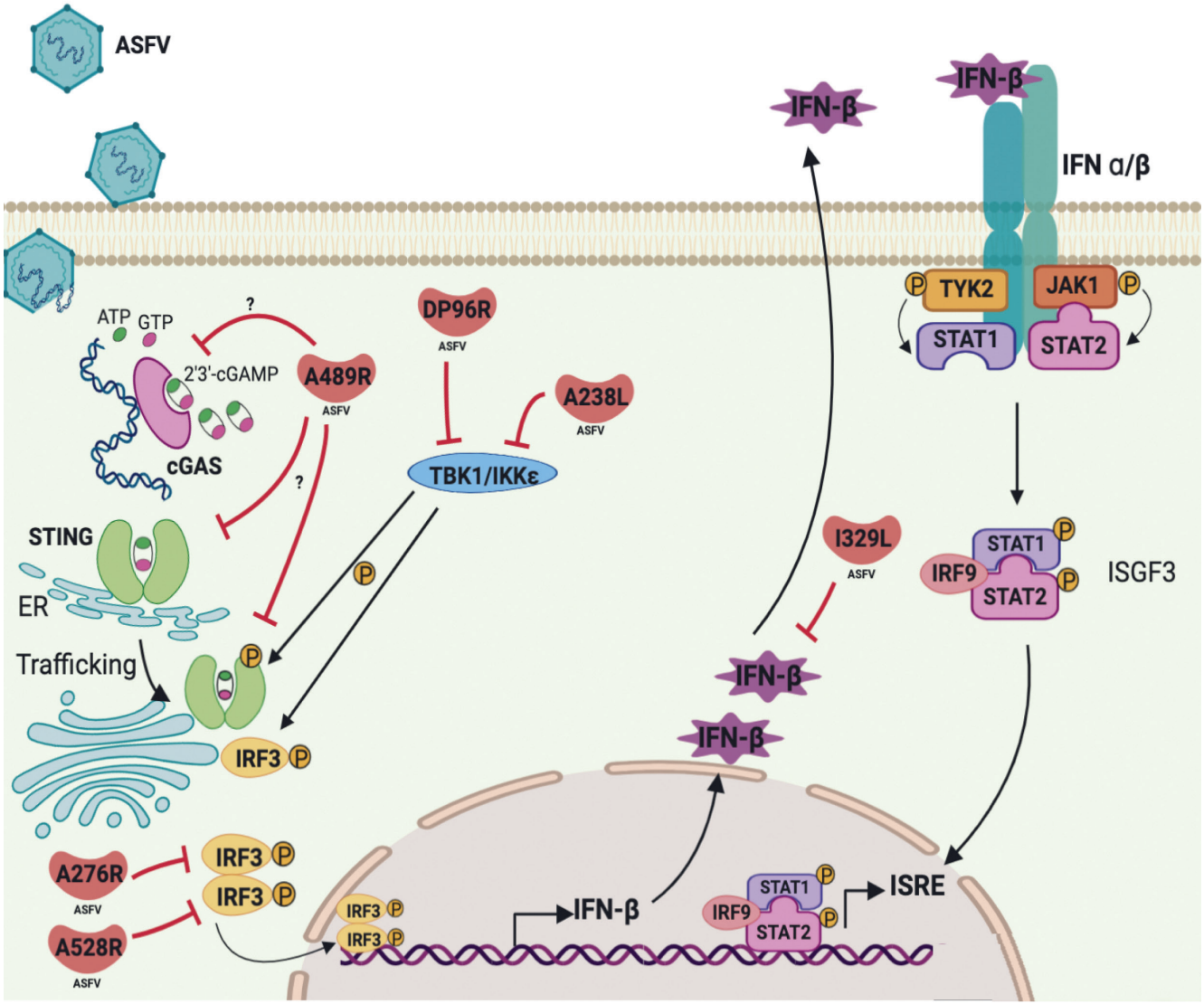
**COORDINATOR**: Dr. Yolanda Revilla, Centro Biología Molecular Severo Ochoa (CBM)-CSIC, Spain
**PARTNERS:** LMU, Germany | NVRI, Poland | SVA, Sweden
**PROJECT PERIOD**: March 2021 – February 2024

### HIGHLIGHTS/MAJOR ACHIEVEMENTS

In the IFNASF Project we have identified a number of African swine fever virus (ASFV) genes potentially involved in type I IFN by in silico screening and scRNAseq technology, validated by in vitro assays. From this information, the best candidates were selected to generate recombinant MVA expressing these genes, and recombinant ASFVs (rASFVs) where these genes were individually deleted. The best rASFV candidate, according to in vitro tests, was used for an in vivo assay where its attenuation was verified, as well as its ability to induce protection against a virulent isolate. An important link between type I IFN control and ASFV virulence is thus established.

### PERSPECTIVES AND RECOMMENDATIONS FOR FUTURE ACTIVITIES

The aforementioned recombinant viruses generated, both MVAs and ASFVs, are key tools for the study of the function of these ASFV genes identified in this project involved in type I IFN control. But also, and importantly, at least in those tested in vivo that have proved to be safe, for their study in their involvement in virulence. Moreover, they constitute in themselves tools for safety in strategies based on subunit vaccines (MVAs) or based on live attenuated vaccines (LAVs) in the case of recombinant ASFV. In the near future some of these candidates will be used as templates for the generation of new generation ASFV vaccines.




The diagram shows the cGAS/STING pathway, which leads to type I IFN production, and the JAK/STAT pathway, which amplifies the type I IFN signal, both of which are controlled by ASFV during infection. Only some of the ASFV proteins controlling some of the steps are shown. Our project highlights the importance of identifying new ASFV genes involved in the control of these pathways, as this is key to identifying new virulence factors.

## PUBLICATIONS IN PEER REVIEWED INTERNATIONAL JOURNALS

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# CAE-RAPID

## Development of a rapid screening test for on-site serological diagnostics of caprine arthritis-encephalitis using individual milk samples

**COORDINATOR:** Prof. dr. hab. Michał Czopowicz, Institute of Veterinary Medicine, Warsaw University of Life Sciences-SGGW, Poland
**PARTNERS**: NMBU, Norway | UNIBE-IVI, Switzerland | UVMB, Hungary | LSMU, Lithuania
**PROJECT WEBSITE:** https://tiny.pl/h88pmh70
**PROJECT PERIOD:** March 2021 - January 2025

### HIGHLIGHTS/MAJOR ACHIEVEMENTS

- An immunochromatographic lateral-flow rapid test for detection of antibodies to small ruminant lentivirus (SRLV) has been developed.
- The test can detect antibodies within 30 min. in whole blood, serum, and milk.
- The test has high diagnostic sensitivity (~80%), very high diagnostic specificity (~99%), and is very highly reliable.
- Epidemiological situation of caprine arthritis-encephalitis (CAE) in Hungary and Lithuania has been investigated for the first time using both serological and molecular tests.


The CAE-RAPID immunochromatographic lateral-flow test in action.

### PERSPECTIVES AND RECOMMENDATIONS FOR FUTURE ACTIVITIES

The CAE-RAPID project has provided important conclusions regarding rapid diagnostics of SRLV infections in small ruminants. It clearly shows that it is possible to develop a rapid serological test that will allow a veterinarian or a farmer to find out if an animal is SRLV-infected with fairly high certainty and without a need to send biological samples to a laboratory. The rapid test will soon be patented with prospect of successful commercialization. However, there is still an area for improvement of the rapid test diagnostic performance associated mainly with antigenic composition of the test. Not all viral antigens commonly used in laboratory serological tests such as ELISAs appear to fit immunochromatographic format. Certainly, they need advanced modifications to stick to the lateral-flow membrane and not to block the flow of reagents. Developing new combinations of viral antigens might further increase diagnostic sensitivity of the rapid test without losing its enormously high specificity.

Epidemiological studies carried out within the frame of the CAE-RAPID project have shown for the first time that SRLV infection is widespread in Hungary and Lithuania and various genetic variants of the virus circulate in goat populations of these countries. As a result, intensive actions should be taken in the future to develop effective control strategies for these countries and the results of the CAE-RAPID project will undoubtedly help decision-makers in these countries. Moreover, knowing that SRLV easily crosses interspecies barrier between goats and sheep, further studies should also include sheep populations to provide the most comprehensive view of the epidemiological situation.

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# MUSECoV

## Multi-Scale Eco-evolution of CoronaViruses: from surveillance toward emergence prediction

**COORDINATOR:** Pr S. Le Poder, , INRAE, France

**PARTNERS:** UMR Virologie (S. Le Poder), France | ANSES Ploufragan, France | ANSES Nancy LRFSN, France | Universite de Caen, GRAM, France | MCB, Jagiellonian University, Poland | PIWet; ICN2, Nanobiosensors and bioanalytical applications group, Spain | IZS, Italy | University of Bari, Italy

**PROJECT WEBSITE**: https://umr-1161-virologie.jouy.hub.inrae.fr/research/projects/musecov

**PROJECT PERIOD**: March 2021 – February 2025

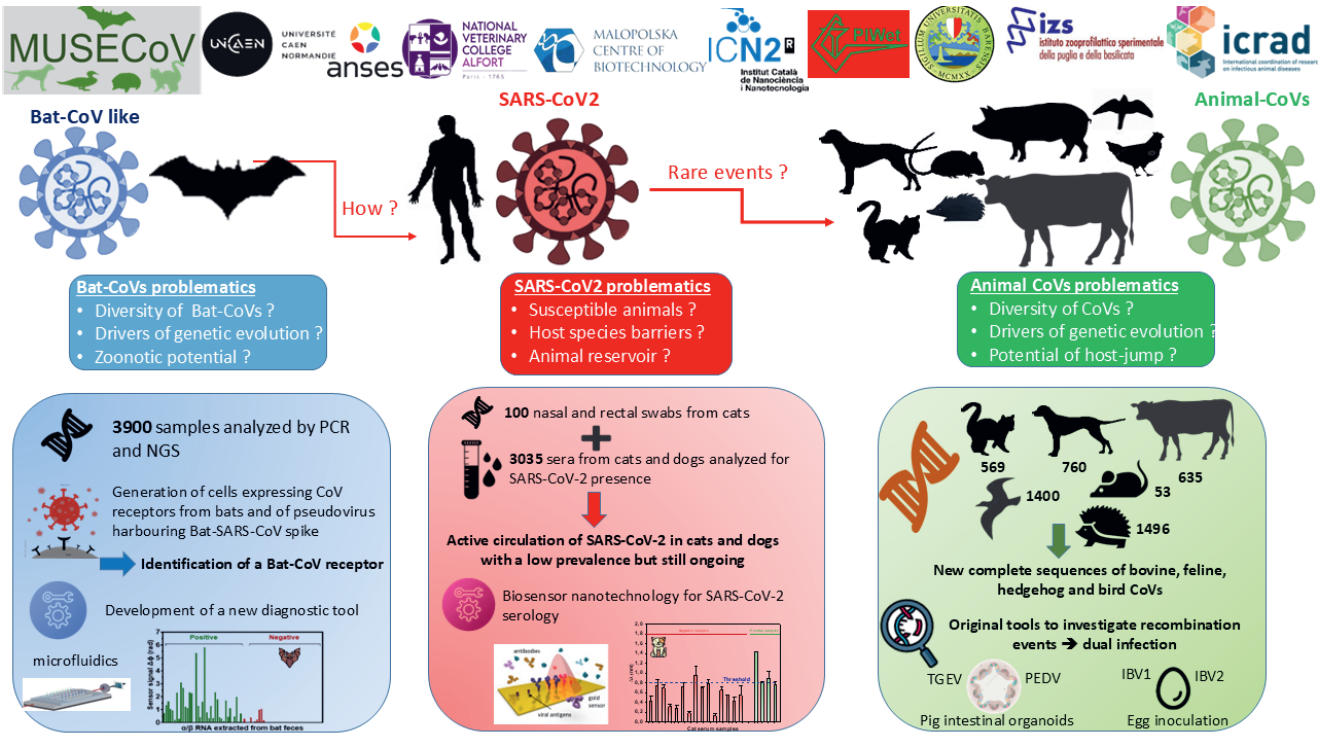
### HIGHLIGHTS/MAJOR ACHIEVEMENTS

Coronavirus (CoV) infections are important diseases that can affect humans, livestock, pets and wildlife. They can evolve by various genetic mechanisms, sometimes allowing them to acquire new biological properties, such as a change in virulence or the ability to infect a new host. The emergence of SARS-CoV-2 is a paradigm of the ability of coronaviruses to cross species barriers. Our global objective was to improve knowledge of the ecology of CoVs to better understand their infection dynamics in different animal populations, and thus the rapid emergence of particularly pathogenic variants. Thanks to the MUSECoV project, we succeeded:

- To collect and analyze around 12000 samples from bats, wild birds, hedgehogs, bovines,dogs, cats, rodents
- To evaluate the ongoing circulation of SARS-CoV-2 in companion animals
- To obtain new full-genome CoVs sequences from bats, hedgehogs, wild birds, cats, bovine
- To identify the genetic evolution over several years and recombination events of a French Bat-CoV
- To develop innovative diagnostic tools for rapid serological and PCR analysis
- To identify the receptor of a Bat-SARS-CoV-like circulating in France

### PERSPECTIVES AND RECOMMENDATIONS FOR FUTURE ACTIVITIES

Thanks to the MUSECoV project, we brought together a unique European consortium of experts with a range of skills, including biotechnology, ecology, genetics and cell cultures. Given the increase in human cases of SARS-CoV-2 in Asia today, this pandemic is not yet over. It is therefore important to continue monitoring this virus in animals, as this will help us to identify markers of cross-species transmission and prevent the emergence of variants from the animal reservoir. Our results open up new avenues for the surveillance of animal coronaviruses (CoVs) and help us to understand the mechanisms by which these zoonotic viruses jump between species. We will further characterise the biological properties of bat coronaviruses that could have zoonotic potential. However, continuing animal coronavirus research is also crucial to identifying emerging zoonotic strains, enabling early intervention and reducing the risk of global outbreaks. This area of research is important for strengthening the public health response and emergency preparedness.

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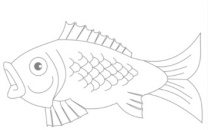
# NucNanoFish

## Nucleic Vaccine for Fish



**COORDINATOR:** Dr Bernard Verrier. Laboratory of tissue biology and therapeutic engineering. UMR5305, University of Lyon, France
**PARTNERS:** INRAE, France | University of Liege, Belgium | University of Aberdeen, United Kingdom | Norwegian University of Life Sciences & Paraclinical Sciences, Norway | Quantoom Univercells, Belgium
**PROJECT WEBSITE**: https://lbti.ibcp.fr/?page_id=4992
**PROJECT PERIOD**: January 2021 - January 2025

### HIGHLIGHTS/MAJOR ACHIEVEMENTS

The NucNanofish objective was to develop nucleic vaccines for fish with a strong focus on mRNA vaccines. We have compared four different viruses and three different fish species (Rainbow trout, Carp and Salmon) and two different mRNA delivery systems, one based on a solid core surrounded by lipids corona, the second one based on lipid only (LipoNanoParticles, (LNP) same tools used in human). We found that LNP was adapted for trout, but not yet optimal for salmon or carp. However, by using a specific mRNA vaccine antigen (encoding VHSV glycoprotein), we bring the first proof of concept that a mRNA vaccine using a LNP carrier and intramuscular route could protect rainbow trout from high dose of virus challenge. This mRNA vaccine was as efficient as an attenuated virus proving that mRNA vaccine could be designed for fish species. However, it was not the case for salmon due either to the choice of a less efficient antigen vaccine candidate and/or a weak efficiency of LNP delivery system. These data illustrate that it exists room for improvement for designing mRNA vaccines and each mRNA vaccine need to be fine-tune according to each fish species which a strong influence of water temperature.

### PERSPECTIVES AND RECOMMENDATIONS FOR FUTURE ACTIVITIES

NucNanoFish project provided the first proof of concept that mRNA vaccine for fish is feasible. It opens new paradigm for designing innovative fish vaccines and offers new vaccine tools for the current challenges faced by aquaculture with global warming and the emergence of new pathogens. As key parameters and methodology have been identified, it is of importance to pursue some research in fish mRNA vaccine as this technology is evolving every, day thanks to IA (innovative mRNA delivery system, mRNA design, improvement of safety, route of administration). Furthermore, concerning One Health perspective and importance of aquaculture, having a task force on mRNA vaccine dedicated to fish mRNA vaccines against main farm fish pathogens and able to produce in a short period of time such nucleic vaccines will permit to cope a potential fish pandemia affecting fish production.

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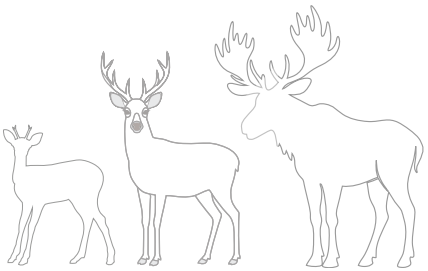
# TCWDE

## Tackling chronic wasting disease in Europe



**COORDINATOR:** Dr Fiona Houston, The Roslin Institute, University of Edinburgh, United Kingdom
**PARTNERS:** NVI, Norway | SVA, Sweden | FLI, Insel Reims, Germany | INRAE, France | CSIC-INIA, Spain
**PROJECT PERIOD:** March 2021 – January 2025

### HIGHLIGHTS/MAJOR ACHIEVEMENTS

Our aim was to better understand the risks of emergent CWD in Europe, both in its capacity to spread rapidly in wild and captive/semidomesticated cervid populations and its potential to threaten livestock and human health. Epidemiological analysis showed large regional variations in confidence of freedom from CWD but indicated that there was unlikely to be a high prevalence of CWD in reindeer. However, the discovery of two additional reindeer cases outside the original culled-out region indicate that the disease has not been eradicated. In contrast, CWD in moose appears to occur as sporadic cases in aged individuals, therefore further spread is unlikely. Analysis of PRNP sequence diversity in wild deer supports the conclusion that the majority of European cervids are probably susceptible to known CWD strains. Two in vitro assays were developed to test the effect of novel cervid PRNP variants on CWD susceptibility, with potential to reduce/replace the need for animal experiments. Parallel transmission to transgenic mice and in vitro experiments show that sheep and cattle are potentially more at risk than pigs from CWD. There was little evidence that European CWD isolates are likely to be directly transmissible to humans, but zoonotic potential of CWD may be enhanced following transmission to an intermediate species (sheep).

### PERSPECTIVES AND RECOMMENDATIONS FOR FUTURE ACTIVITIES

CWD strains identified in Scandinavia are distinct from those found in North America, thus their pathogenic potential cannot be inferred from previous research. Although reindeer CWD was at low prevalence during the study period, it is likely to continue to spread. CWD in Nordic moose appears to be sporadic and non-transmissible, but unusual levels of strain diversity in these cases add to unpredictability of potential outcomes. Mouse bioassays suggesting that sheep and cattle may be susceptible to CWD, and that adaptation of CWD in another species could enhance its zoonotic potential, underline the importance of further research to examine the probability of natural transmission, and continued surveillance for novel forms of prion disease in wildlife, livestock and humans. Comprehensive molecular and pathological strain characterization is essential to delineate the diversity of CWD strains present in Europe, facilitating robust risk assessments and development of targeted control measures.

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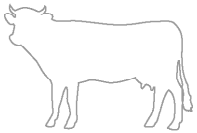
# FMDV_PersIstOmics



## From proteogenomic host response signatures of persistent foot-and-mouth disease virus (FMDV) infection to diagnostic markers and therapeutic control

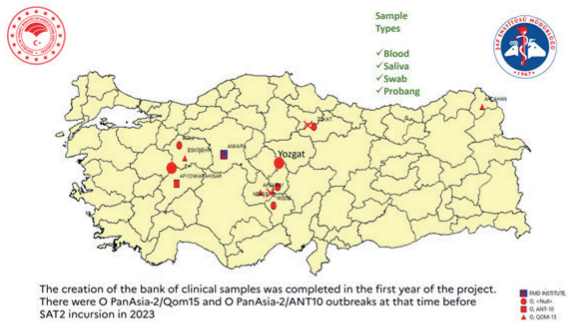
**COORDINATOR:** Dr Sandra Blaise-Boisseau, ANSES, Laboratory for Animal health, Joint research unit of Virology, BIOPIC team, Maisons-Alfort, France
**PARTNERS:** Sciensano, Belgium | FLI, Germany | SAP Institute, Turkey | SLU, Sweden | ANSES, France
**PROJECT WEBSITE:** https://umr-1161-virologie.jouy.hub.inrae.fr/research/projects/fmdv_persistomics#
**PROJECT PERIOD:** March 2021 – January 2025

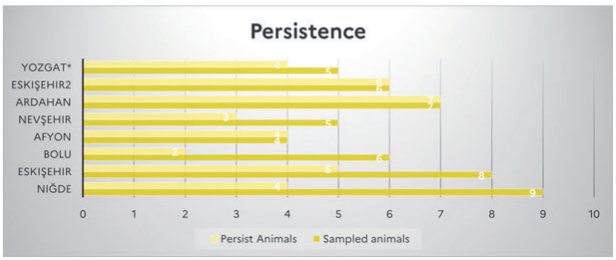
### HIGHLIGHTS/MAJOR ACHIEVEMENTS

Foot-and-mouth disease (FMD) is one of the most contagious and devastating viral diseases of cloven-hoofed livestock. More than 50% of ruminants exposed to FMDV, even vaccinated, develop a persistent infection wherein the virus remains in the nasopharynx for a prolonged period. This "carrier state" presents significant challenges for disease control and eradication but the remaining mechanisms remain to elucidate. In WP1, a transcriptomic analysis workflow was optimized to investigate selected nasopharyngeal samples from cattle experimentally FMDV infected. Differential gene expression analysis identified key candidate genes associated with FMDV persistence. Additionally, sequencing of oropharyngeal fluid samples revealed a set of viral genomic mutations occurring in the persistent phase of infection. The role of the FMDV leader protein in persistence was also investigated through in vitro and in vivo experiments, demonstrating that this protein is essential for establishing a productive infection and viral persistence. Transcriptomic and pathway analyses confirm FMDV persistence in nasopharyngeal lymphoid tissue, with immune evasion linked to in follicle-associated epithelium marker overexpression, epithelial integrity loss, and suppressed antiviral responses. In WP2, a FMDV sample biobank was created, consisting of 78 OPF samples collected from 39 naturally infected cattle during six FMDV outbreaks in Turkey in 2021. Among these cattle, 64% were found carrier. A multiplex reverse transcription real-time PCR assay was developed to target two candidate host markers of FMDV persistence, alongside an epithelial marker for quality control with the aim to obtain a more sensitive method for identifying carrier animals than direct viral detection. The results obtained in WP3 demonstrated that interference with type I IFN signaling is crucial for viral persistence.

### PERSPECTIVES AND RECOMMENDATIONS FOR FUTURE ACTIVITIES

The outcomes of this collaborative project provide valuable insights into the mechanisms of FMDV persistence and the factors influencing it. The findings will contribute to improving diagnostic methods, enhancing disease control strategies, and developing potential interventions to mitigate the carrier state, ultimately aiding in the global effort to control and eradicate FMD. These findings are definitely in line with the come back of FMD in Europe since January 2025 and efforts will be continued under the EUPAHW 2026-2028.




***Overall 25 probang samples out of 39 collected after 35 days were detected positive, representing 64% of animals persistently infected by FMDV***

**Creation of a bank of positive clinical samples (n=20 to 25) collected from persistently infected cattle task <u>has been achieved</u>**
***<u>(Manuscript in preparation)</u>***

A bank of positive clinical samples (n=20 to 25) collected from persistently infected calves.

## PUBLICATIONS IN PEER REVIEWED INTERNATIONAL JOURNALS

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# PIGIE

## Understanding the dynamics and evolution of swine influenza viruses in Europe: relevance for improved intervention and sustainable pig production

**COORDINATOR:** Dr. Gaëlle Simon, ANSES, Ploufragan-Plouzané-Niort Laboratory, Swine Virology Immunology Unit, France
**PARTNERS:** ANSES, France | FLI, Germany | UCPH, Institute for Veterinary and Animal Sciences, Denmark | IZSLER-Parma, Italy | UAB, Sanitat i Anatomia Animals, Spain | APHA, Virology, United Kingdom
**PROJECT PERIOD:** March 2021 – September 2024

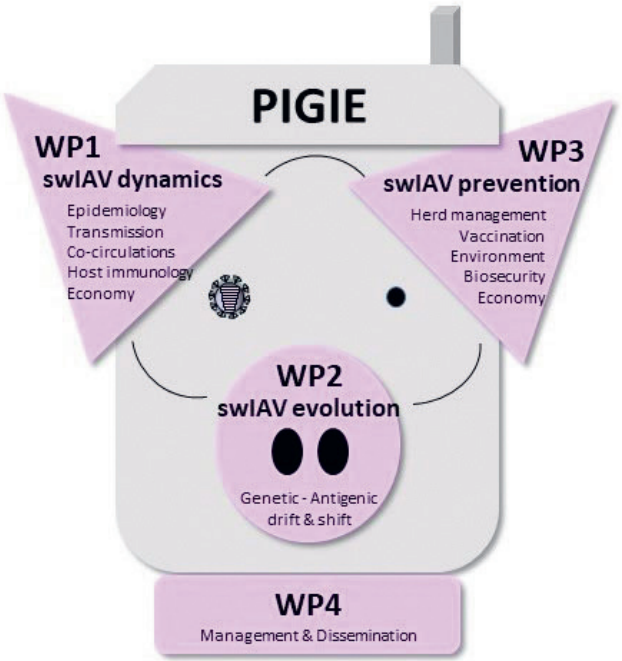
### HIGHLIGHTS/MAJOR ACHIEVEMENTS

This project aimed to better understand and manage swine influenza A virus (swIAV) in large pig herds with permanent infections across several European countries. The main objectives were to identify the factors influencing disease prevalence, assess the impact of enzootic swIAV infections on animal welfare, production, and economic productivity, and analyze the virus's genetic and antigenic diversity in Europe.

Sampling from selected herds showed a high proportion of swIAV-positive pigs, with age-group-specific variation in infection dynamics. SwIAV infections often occurred during the late farrowing phase or shortly after weaning, with prolonged shedding or re-infections observed in individual piglets. Detected subtypes included H1 viruses belonging to HA-clade 1A, 1B and 1C, with mixed or successive infections reported in half of the farms. Co-infection with other respiratory pathogens was also common. A reference framework for swIAV genotypes from 2012–2022 was established, alongside a genotyping nomenclature system. Regional diversification was observed, particularly due to reassortments. HA-1B.1.1 was limited to England, while antigenic drift was notable among HA-1A strains, varying between countries. HA-1C viruses exhibited the highest antigenic diversity, especially within HA-1C.2.4 strains, which differed significantly from the HA-1C.2.2 vaccine antigen. The study also investigated the immunological memory responses in infected hosts, including maternally-derived and post-infectious immunity, revealing a complex relationship between host and virus factors. Control strategies, including biosecurity improvements, herd management, and tailored farm modeling, were implemented to combat sustained infections. Reducing airflow and animal movement were identified as key factors in limiting swIAV persistence. Vaccination showed promise when using recombinant platforms with autologous isolates, whereas general vaccines failed to ensure full protection.

### PERSPECTIVES AND RECOMMENDATIONS FOR FUTURE ACTIVITIES

Unlike snapshot surveillance, longitudinal sampling proved crucial for understanding swine influenza dynamics, supporting better control strategies and vaccine deployment. The PIGIE sequence database offers a valuable overview of European genetic diversity, aiding risk assessment. Stakeholders can use findings from genetic and antigenic studies, vaccination trials, and modelling to enhance control measures. PIGIE also emphasized the importance of systems-based approaches to deepen understanding of swine influenza and reduce zoonotic risk.

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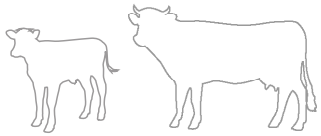
# PLANTS4NEMAVAX



## Plant-based production of glyco-engineered nematode vaccines

**COORDINATOR**: Prof Dr Peter Geldhof, Laboratory of Parasitology, Faculty of Veterinary Medicine, Ghent University, Belgium
**PARTNERS:** Moredun Research Institute, United Kingdom | Wageningen University, The Netherlands | Leiden University Medical School, The Netherlands
**PROJECT PERIOD:** April 2021 – March 2024

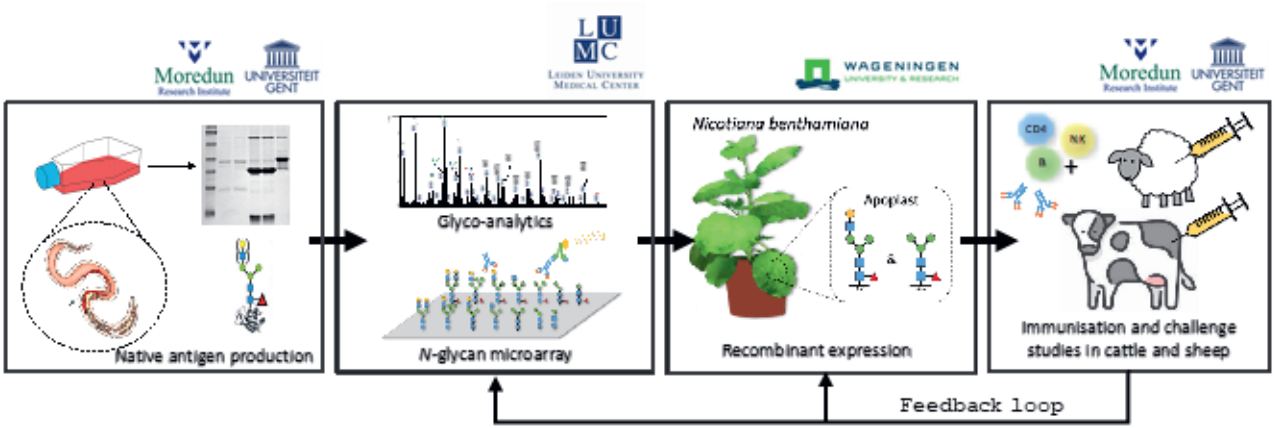
### HIGHLIGHTS/MAJOR ACHIEVEMENTS

Parasitic nematodes are amongst the most common pathogens in both humans and animals. Control of worm infections currently relies heavily on the use of anthelmintic drugs. However, with the increasing incidence of anthelmintic resistance, there is an urgent need for alternative control measures. Vaccination is regarded as the most rational and cost-effective alternative. Although effectiveness of anti-parasitic vaccines has been demonstrated repeatedly, there are hardly any commercial vaccines currently available. A major hurdle has been the production of recombinant vaccines in heterologous expression systems and in particular the inability of the expression systems to reconstitute the antigens with their native N-glycans. These N-glycans are often nematode specific and highly immunogenic. However, scientific evidence to confirm that N-glycans are truly important in the immunoprotective capacity of nematode vaccine antigens was largely missing.

Through the Plants4nemavax project we been able for the first time to provide scientific evidence that (1) the N-linked glycans present on two native vaccine antigens from two parasite species infecting cattle play a role in the vaccine induced immune response and (2) that the reconstruction of the natural glycans on the recombinant antigens in a plant-based expression system improves their immunogenicity. The workflow that was developed to achieve this scientific milestone is schematically depicted in the illustration.

### PERSPECTIVES AND RECOMMENDATIONS FOR FUTURE ACTIVITIES

The results obtained in this project form an important breakthrough in this research area as it provides proof-of-concept that efficacious recombinant anti-worm vaccines can be produced if glycans are considered properly. Nevertheless, the precise mechanisms underlying this process are still incompletely understood. Future research is therefore needed to better understand which glycan residues contribute to the immunogenicity of vaccine antigens and how this can influence antibody induction and - binding.




Workflow designed to produce glycan-engineered vaccine antigens and test their immunostimulatory and protective capacities in sheep and cattle.

## PUBLICATIONS IN PEER REVIEWED INTERNATIONAL JOURNALS

Plant based production of a protective vaccine antigen against the bovine parasitic nematode Ostertagia ostertagi. Zwanenburg L., Borloo J., Decorte B., Bunte M.J.M., Mokhtari S., Serna S., Reichardt, N.C., Seys L.J.M., van Diepen A., Schots A., Wilbers R.H.P., Hokke C.H., Claerebout E., Geldhof P. Scientific Reports 2023 Nov 22;13(1):20488. doi: 10.1038/s41598-023-47480-3

Identification of beta-galactosidases along the secretory pathway of Nicotiana benthamiana that collectively hamper engineering of galactose-extended glycans on recombinant glycoproteins. Van der Kaaij A., Bunte M., Nijhof L., Mokhtari S., Overmars H., Schots A., Wilbers R.H.P., Nibbering P. Plant Biotechnology Journal 2025 May 7 doi: 10.1111/pbi.70126

# ConVErgence

## Assessing swine as potential hosts for emerging Coronaviruses

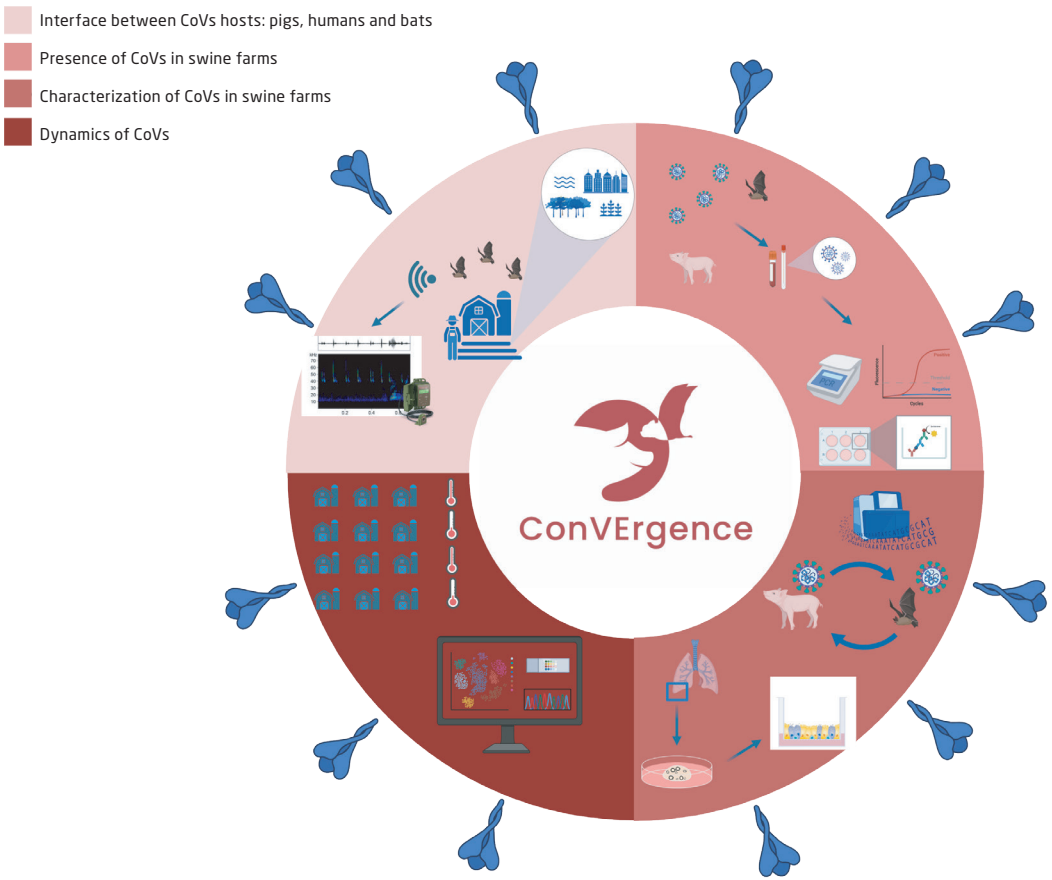
**COORDINATOR:** Paola De Benedictis, Istituto Zooprofilattico Sperimentale delle Venezie, Italy
**PARTNERS:** Erasmus Medical Centre – Viroscience, The Netherlands | University of Sussex, United Kingdom
**PROJECT WEBSITE**: https://www.izsvenezie.com/convergence-project-swine-cornaviruses
**PROJECT PERIOD:** March 2021 – November 2024

### HIGHLIGHTS/MAJOR ACHIEVEMENTS

ConVErgence aimed at investigating bat-pig and human-pig interfaces to predict and identify risks for Coronaviruses (CoVs) emergence in pig farms. Considering the bat-pig interface, we found no evidence for silent circulation of bat CoVs in pigs. Failure to wear facemasks was identified as a significant risk for human-to-pig spillover events, especially considering that the fieldwork was performed during the peak of COVID-19. Similar to what was found for bat CoVs, we found no evidence of SARS-CoV-2 circulation among pigs in Italy or the Netherlands using molecular and serological analyses, confirming that they do not currently play a role in the maintenance or amplification of this pandemic virus. We also investigated whether SARS-CoV-2 can infect pigs using classical cell lines and organoids. We support the ability of SARS-CoV-2 to interact with swine receptors but proved ACE-2 is not exposed in the cilia of pig airway cells, hampering the infection at this level. This peculiarity is due to a mutation in the gene coding for pigs ACE-2, and plays the role of a shield to the infection not only with SARS-CoV-2 but also with other viruses using this receptor for the infection, including other bat CoVs.

### PERSPECTIVES AND RECOMMENDATIONS FOR FUTURE ACTIVITIES

Although we did not detect any spillover events, our screening showed a high prevalence and frequency of endemic CoVs in swine herds. These viruses are rarely screened, sequenced and studied because they do not generally cause severe clinical disease. However, we confirmed the growing evidence worldwide that these viruses are evolving by chancing their tropism. Indeed, our strains showed deletions in the S gene of PRCV (and alphaCoV) and the almost complete deletion of NS2 gene of PHEV (a betaCoV). These mutations are considered to be responsible for the increased respiratory tropism of both viruses. Since these mutations could also influence their ability to infect hosts other than swine, we suggest not to neglect them and monitor them in a One Health perspective.

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Drzewnioková, P.; Festa, F.; Panzarin, V.; Lelli, D.; Moreno, A.; Zecchin, B.; De Benedictis, P.; Leopardi, S. Best Molecular Tools to Investigate Coronavirus Diversity in Mammals: A Comparison. Viruses 2021, 13, 1975. https://doi.org/10.3390/v13101975

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# BM-FARM

## Biomarkers and Microbiome in Farms for Antimicrobial Resistance Management

**COORDINATOR:** Edgar Garcia Manzanilla, Pig and Poultry Research and Knowledge Transfer Department, Teagasc, Moorepark, P61KX38, Cork, Ireland
**PARTNERS:** UCD, Ireland | INRAE, France | UMU, Spain
**PROJECT WEBSITE:** https://www.teagasc.ie/animals/pigs/research/research-projects/bm-farm-project
**PROJECT PERIOD:** March 2021 – November 2024

### HIGHLIGHTS/MAJOR ACHIEVEMENTS

BM-FARM described, for the first time, the associations between the characteristics of pig commercial farms (i.e. productive performance, management practices, health and welfare status), the intestinal microbial populations and a wide array of physiological biomarkers present in pigs from birth to slaughter and using a cohort of commercial farms.

The main factor shaping the intestinal microbiome during weaning was the use of in-feed antibiotics and zinc oxide. After weaning, the type of diet (solid vs liquid) was the main factor affecting the microbiome. The physiological biomarkers were severely altered by the weaning process showing changes related to inflammation and stress. After weaning, most biomarkers were normalized and remained stable despite changing facilities at different stages.

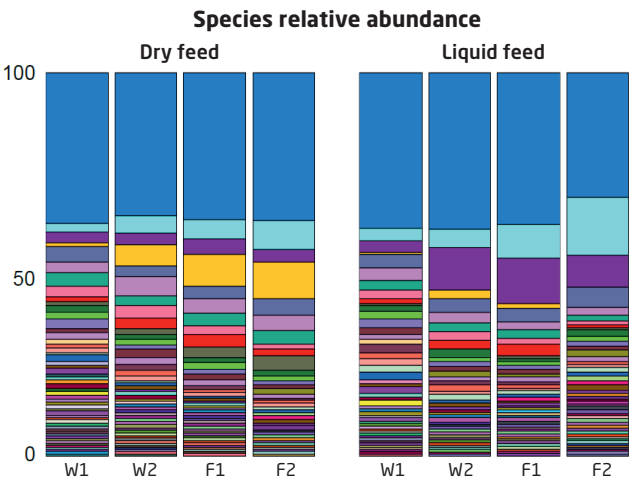
Hygiene and co-mingling during lactation were tested in commercial farms as interventions to minimize the effects of weaning in the pig. The effects of hygiene and co-mingling on the intestinal microbiome and biomarkers was less marked than expected despite having clear effects on the health and welfare of the pigs (less diarrhea and lesions).

Finally, BM-FARM has achieved significant progress in the area of oral fluid biomarkers by refining existing techniques and developing analysis for different analytes in oral fluids of pigs like procalcitonin or myeloperoxidase. In a parallel activity, BM-FARM has described the changes in a wide panel of biomarkers in pigs with S. suis meningitis.

### PERSPECTIVES AND RECOMMENDATIONS FOR FUTURE ACTIVITIES

The intestinal microbiome responded well to factors targeting the gastrointestinal tract like oral antibiotics or changes in the diet, but it did not reflect other major changes in systemic health and welfare. The microbiome of other parts of the pig needs to be investigated. The oral, respiratory and reproductive microbiome seem to be the next ones to target.

Oral fluid biomarkers have shown potential for field use in pigs, and they need further research, especially higher number of farmers to narrow and better define the physiological thresholds and conditions for use.




Changes in the microbiome of pigs along their life depending on the type of feed received. Each colour represents a core microbiota species, with darker blue bars on top aggregating low-abundant species (<5%).

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# ASF-RASH

## African Swine Fever pathogenesis and immune responses in resistant and susceptible hosts

**COORDINATOR**: Dr. Sandra Blome, Institute of Diagnostic Virology, FLI, Germany
**PARTNERS:** FLI, Germany | IVI, Switzerland | WBVR, The Netherlands | Ghent University, Belgium | Sciensano, Belgium | SSI, Denmark
**PROJECT PERIOD:** March 2021 – February 2024

### HIGHLIGHTS/MAJOR ACHIEVEMENTS

- Maternal immunity via colostrum or serum transfer did not protect piglets from lethal ASFV infection.
- ASFV was transmitted via semen; infected boars shed virus as early as 2 days post-inoculation, causing infection and reproductive failure in gilts.
- Long-term studies showed that pigs surviving infection with moderately virulent ASFV can develop solid clinical protection against highly virulent challenge
- Comparative trials confirmed high virulence of recent German ASFV strains, almost matching that of Armenia 2008, while Estonia 2014 remained moderately virulent
- Systems immunology identified early, controlled IFN-alpha responses as key protective correlates, with prolonged inflammation linked to poor outcomes.
- We were able to confirm that African suids—red river hogs and warthogs—are completely resistant to clinical ASF despite infection with a highly virulent ASFV strain that killed European wild boar and domestic pigs within less than a week. African species exhibited low viraemia, limited viral distribution in organs, and a controlled immune response, unlike the strong inflammatory reaction seen in European pigs.
- Macrophages from Tayassuidae (peccaries) were resistant to ASFV, unlike those from all tested Suidae, which were susceptible.
- Using nasal explants, it was shown that ASFV can infect multiple cell types in the respiratory tract, with genotype-specific preferences for epithelial entry routes. A novel vein explant model revealed that ASFV primarily targets perivascular macrophages. Comparative replication studies in primary macrophages showed that virulence is not reflected in vitro.
- Transcriptomic profiling revealed key differences between porcine nasal and lung macrophages. Nasal macrophages showed higher immune activation and niche-specific adaptation, while lung macrophages had classic immune profiles.
- Genomic analysis of ASFV strains revealed strain- and host-specific variants, underscoring high genetic variability and host adaptation potential.

### PERSPECTIVES AND RECOMMENDATIONS FOR FUTURE ACTIVITIES

Our findings on maternal immunity, immune duration, correlates of protection, and infection in distinct cell types—including insights from African wild suids—should guide vaccine design and control strategies. Future studies should apply advanced tools like systems immunology and single-cell RNAseq. Monitoring of boars and semen is critical, as ASFV can be transmitted via semen. Continued genome surveillance remains essential to detect emerging variants and support timely adaptation of diagnostic, preventive, and control measures.

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**CALL 2:**

# One Health approach to zoonoses research and innovation

The overall objective is to increase preparedness to (re)-emerging zoonotic diseases and ability to respond to zoonotic threats and contribute to improved animal and public health.

Research and innovation funded through this call should seek a concerted approach towards the development of novel and/or improved instruments to understand and control zoonoses, including detection, management, intervention and prevention strategies.

# LEPTIMMUNHOST

## Comparative host and species-specific immune responses of macrophages infected with zoonotic Leptospira interrogans

**COORDINATOR:** Catherine WERTS, Institut Pasteur, Innate immunity and Leptospira group, France
**PARTNERS:** The Royal Veterinary College, United Kingdom | INTA/CONICET, Argentina | Vilnius University, Lithuania
**ASSOCIATED PARTNER:** University of Victoria, Canada
**PROJECT PERIOD:** July 2023 – June 2026

### HIGHLIGHTS/MAJOR ACHIEVEMENTS

*Leptospira interrogans* are bacteria that causes leptospirosis, an emerging zoonotic disease affecting humans and animals worldwide. The disease can present as an acute, potentially fatal infection in accidental hosts, such as humans or hamster, or progress into a chronic, primarily asymptomatic infection in natural hosts, such as mice and rats. Leptospirosis causes high economic losses in livestock, due to morbidity and high abortion rates. We hypothesized that these differences may be due to differential sensing of leptospires by the innate immune system. Specifically, we aimed to compare the recognition of leptospires and cell wall components by Toll-like receptors (TLR), using structural, biochemical, genomic, immunological and computational modeling approaches.

We constructed expression vectors for bovine, equine, porcine, human, mouse and hamster TLR4/MD2/CD14, TLR2, and TLR5, which recognize lipopolysaccharides (LPS), lipoproteins, and flagellins, respectively. We then compared their recognition of various Leptospira strains in the HEK 293 system. We observed differential responses between hosts and serovars. We analyzed the whole-genome sequences of clinical L. interrogans samples from Argentina and model strains to compare LPS and flagellin loci between serovars. Structural studies of leptospiral ligand-receptor interactions are ongoing.

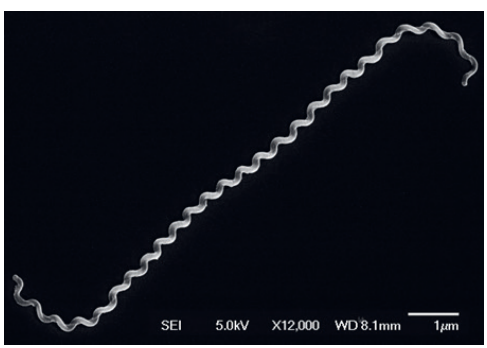
### PERSPECTIVES AND RECOMMENDATIONS FOR FUTURE ACTIVITIES

We will study the leptospiral recognition by the rat's TLRs, because rats are the main reservoir for *L. interrogans* strains causing severe infections. Additionally, we will examine leptospiral binding to C-type lectin receptors using bovine and human arrays.

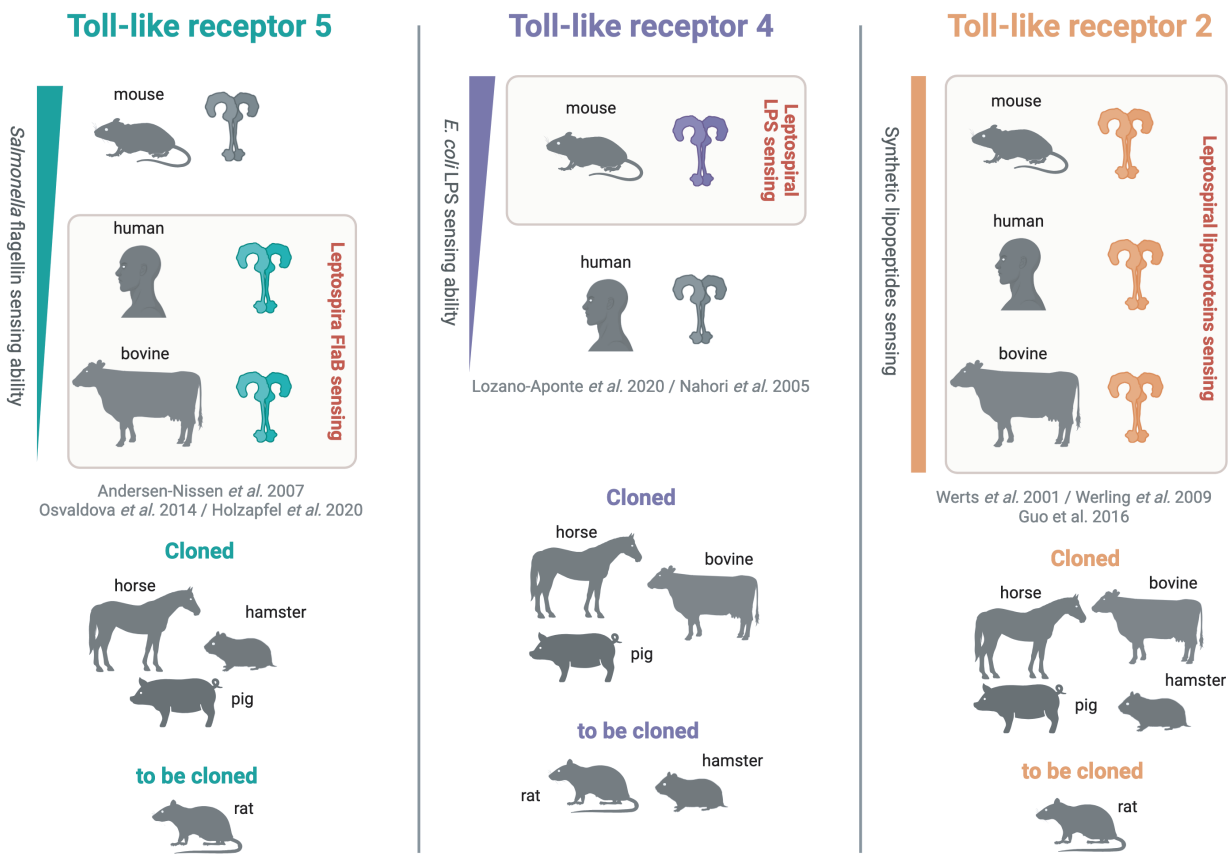
Meanwhile, we will investigate the structures of the different parts of LPS (lipid A and O antigen, the latter known to modulate the Lipid A responses through TLR4) in several strains of *L. interrogans*, grown in culture conditions mimicking the host.

We also studied a mouse model of acute lethal leptospirosis. We determined that, as in humans, the disease caused myocarditis and neutrophilia; the latter is associated with vascular damage. We will study the differential TLR responses in macrophages and neutrophils derived from human blood as well as from bovine, hamster, and mouse bone marrow cells.

This project should help us better understand the innate immune mechanisms that drive host-specificity in leptospirosis. This knowledge could be used to develop host-directed therapies.




Electronmicroscopy of *Leptospira interrogans.*




*LEPTIMMUNHOST TLR project.*

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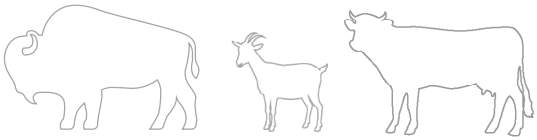
# imdiTBap



## Improving the diagnosis of tuberculosis in domestic ruminants through the use of new antigens and test platforms

**COORDINATOR:** Dr. Javier Bezos, Animal Health Department and VISAVET, Complutense University of Madrid, Spain
**PARTNERS:** APHA, United Kingdom | UCD, Ireland | IZSLER, Italy | IZSM-CReNBuf, Italy | ISCIII, Spain | UC, Turkey
**PROJECT WEBSITE:** https://www.visavet.es/en/research/projects/imdiTBap.php
**PROJECT PERIOD:** April 2023 – March 2026

### HIGHLIGHTS/MAJOR ACHIEVEMENTS

The first 18 months of the project were mainly focused on herd selection (cattle, goats, buffaloes), evaluation of DST-F and P22 in the intradermal test (IT) and interferon-gamma release assay (IGRA), and the creation of a serum and plasma sample bank for validating experimental techniques (such as P22 ELISA and a multic-cytokine detection platform based on Luminex technology) for diagnosis of tuberculosis in domestic ruminants.

Preliminary analysis of more than 2,000 samples suggests that the performance of IT and IGRA using DST-F and P22 antigens may vary depending on epidemiological conditions, animal species, the test used, and the interpretation criteria applied.

Overall, their use shows a better balance between sensitivity and specificity compared to commercial PPDs, in both IT and IGRA, which could influence future usage strategies.

P22 ELISA showed similar performance to the IT test in goats, and the use of milk samples (when available) showed slightly higher reactivity in infected settings compared to serum, suggesting it could be a valuable sample for humoral diagnosis in such contexts.

However, it is still too early to draw definitive conclusions, as studies are currently ongoing.

### PERSPECTIVES AND RECOMMENDATIONS FOR FUTURE ACTIVITIES

Preliminary results, which will need to be confirmed before the conclusion of the project, suggest that the antigens and diagnostic platforms evaluated in the context of the imdiTBap project could be highly useful for improving the diagnosis of tuberculosis in domestic ruminants within future control and eradication programs. Therefore, the transfer of these results to those responsible for designing such programs — an activity planned for the end of the project — will be essential to achieve the goal of supporting tuberculosis eradication under different epidemiological situations.

## PUBLICATIONS IN PEER REVIEWED INTERNATIONAL JOURNALS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# POC4AIV

## Preventing zoonoses by screening Avian Influenza Virus (AIV) in wildlife birds and poultry using a novel rapid point of care system

**COORDINATOR**: Prof. Winnie E. Svendsen, DTU Bioengineering, DTU, Denmark
**PARTNERS:** IZSVe, Italy | NCU, Torun, Poland | BIOR, Latvia | EMU, Estonia | IVBIO, Turkey | ANSES, France | DNA Diagnostics, Denmark
**PROJECT WEBSITE:** https://poc4aiv.dtu.dk
**PROJECT PERIOD:** April 2023 – March 2026

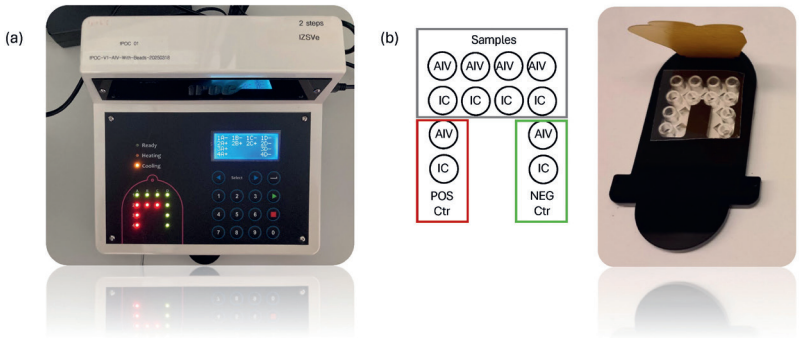
### HIGHLIGHTS/MAJOR ACHIEVEMENTS

A point-of-care device, the fPOC, has been developed for the rapid detection of Avian Influenza (AIV) in poultry, wild birds and their environment. The fPOC uses a novel cartridge with 12 wells that can detect AIV using LAMP (Loop-mediated isothermal amplification) technology. Each cartridge can accommodate 4 samples, as well positive and negative controls. To increase sensitivity a magnetic bead-based sample preparation protocol (MBSPM) has been developed and optimized to decrease the overall time for sample preparation. The results can be obtained in one hour, incl. sample preparation. The fPOC cartridges have a shelflife of 6 weeks.

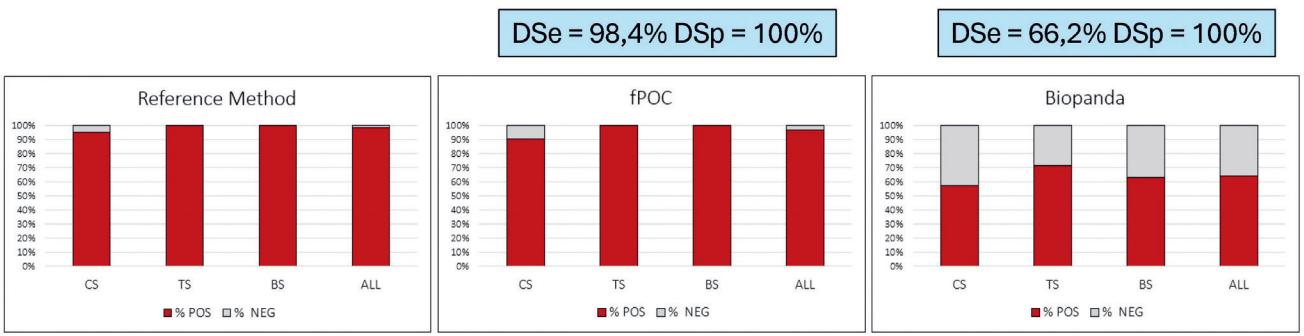
The sample preparation method and the fPOC have been validated in the laboratory using both spiked samples and field samples. The MBSPM has no influence on the Cq values compared to reference methods. The fPOC sensitivity is lower than qRT-PCR at very low viral concentrations, usually lower than 10-100 GC/µl. The inclusivity of the fPOC is 100% tested on 35 AIV strains of different subtypes, incl. H5 and H7. We are currently evaluating the fPOC on field samples and the results so far (with a limited number of samples) indicate a diagnostic specificity (DSp) of 100%, while the diagnostic sensitivity (DSe) is currently between 75%-100% depending on the type of sample. When testing unconventional easy-to-collect samples and comparing the fPOC to the Biopanda lateral flow test, the fPOC shows a DSe of 98.4% and a DSp of 100%, compared to Biopanda's DSe of only 66.2%.

### PERSPECTIVES AND RECOMMENDATIONS FOR FUTURE ACTIVITIES

The great advantage of the fPOC lies in the fast on-site determination of the AI status in wild migrating birds and in poultry flocks. This allows for fast reactions from the authorities to curb disease spread. Besides, mass screening during outbreaks alleviates conventional laboratory pressure and allows for faster lifting of restriction zones and movement of animals. To accommodate this vision, we will in the future automatize the MBSPM, for supporting measurements in the field and extend the shelflife of the cartridge. Moreover, more targets, like H5 HP, can be added in the future.




An image of the fPOC after the end of a run. The green lights indicate a negative sample, while the red light indicates a positive sample. (b) The fPOC cartridge along with a schematic showing the placement of the samples and controls.




Comparison between the reference method (qRT-PCR), fPOC and Biopanda on samples from infected chickens taken on day 1, 2 and 3 post infection. CS: Cloacal swabs, TS: Tracheal swabs, BS: Breast swabs.

## PUBLICATIONS IN PEER REVIEWED INTERNATIONAL JOURNALS

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# EPICVIR

## Emerging porcine influenza and coronaviruses

**COORDINATOR:** Prof. Kristien Van Reeth, Laboratory of Virology, Faculty of Veterinary Medicine, Ghent University, Belgium
**PARTNERS:** Ghent University, Belgium | Utrecht University, The Netherlands | CIB, Spain | Pirbright Institute, United Kingdom | Universidad Pontificia Comillas, Spain | University of Leeds, United Kingdom
**PROJECT WEBSITE:** https://sites.google.com/view/epicvir/home
**PROJECT PERIOD:** September 2023 – August 2026

### HIGHLIGHTS/MAJOR ACHIEVEMENTS

The EPICVIR project aims to compare the transmission dynamics (WP1), pathogenesis and immune control (WP2), and host tropism (WP3) of 6 different swine influenza A virus (swIAV) genotypes, swine influenza D virus (swIDV) and porcine respiratory coronavirus (PRCV). It aims to design an integrated mathematical model (WP4) identifying key events in virus-host interaction and informing future control strategies.

In the first transmission experiments in pigs, nasal virus excretion was one day longer for PRCV than for 2 highly diverse swIAV, the 2009 pandemic H1N1 (pH1N1) virus and the Eurasian avian H1N1 (EA H1N1) virus). All 3 viruses were transmitted between pigs, by direct contact and by airborne contact. Only swIAV were transmitted from pigs to ferrets, which are used as a model for humans. Infectious PRCV and swIAV were also isolated from the environment, mainly from objects in direct contact with the pigs. Transmission experiments with 4 other "reassortant" swIAV genotypes and with swIDV are planned.

Pig infection studies were performed with the 2 above-mentioned swIAVs, swIDV and PRCV. Pigs were euthanized on day 1, 5 and 12 post inoculation (PI). Samples from the respiratory tract, blood, and lymphoid organs were collected to analyse virus replication and pathology as well as innate, antibody, T and B cell responses. PRCV infection induced more severe upper and lower respiratory tract pathology and higher nasal viral shedding compared to pH1N1. Additionally, bronchoalveolar lavage samples from PRCV-infected animals exhibited a greater frequency of interferon-gamma and interleukin-2-producing cells than those infected with pH1N1. We are currently performing RNA sequencing of samples from virus-infected pigs, and of immune cells that have been inoculated with virus in vitro.

In in vitro experiments, swIAVs of 6 different genotypes were compared for their binding to "avian type" and "human type" sialic acid receptors. All viruses seemed to prefer binding to the human-type receptor. Two Eurasian avian swIAVs also showed relatively good binding to avian-type receptors. Further studies to link receptor preference with replication efficiency in cells of human airways are planned.

### PERSPECTIVES AND RECOMMENDATIONS FOR FUTURE ACTIVITIES

Our results will help predict the zoonotic potential, transmission, and pathogenicity of existing and emerging swIVs and PRCVs.

# AdapTB

## Defining the Molecular Determinants of Mycobacterial Adaptation and host:pathogen Interaction to inform bTB control.

**COORDINATOR:** Dr Sharon L Kendall, Centre for Emerging, Endemic and Exotic Diseases, Pathobiology and Population Sciences, Royal Veterinary College, United Kingdom

**PARTNERS**: University College Dublin (UCD), Ireland | National Research Institute for Agriculture Food and the Environment (INRAE-ISP, INRAE-IBIR and INRAE-PFIE), France | National Institute of Agricultural Technology (INTA), Argentina | Birkbeck (BBK), University of London, UK

**PROJECT WEBSITE:** https://adaptb.wordpress.com

**PROJECT PERIOD:** July 2023 – July 2026

### HIGHLIGHTS/MAJOR ACHIEVEMENTS

The eradication of bTB is hindered by a complex heterogenous host response to *Mycobacterium bovis* (Mb). This differential host response results in either complete clearance of the pathogen, sub-clinical disease, or disseminated infection. Onward transmission risk is influenced by the infection outcome; however, we currently have a limited understanding of the pathogen's genetic and host immunological factors influencing disease outcomes. The aim of AdapTB is to fill this knowledge gap.

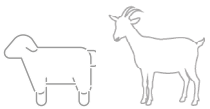
At the mid-term point we have utilized both whole genome and single gene target approaches to identify the pathogen factors required for survival and dissemination of Mb. We have shown that deletion of a regulatory system in Mb AF2122/97 *(phoPR)* is severely attenuated in a murine model (C3HeB/FeJ) known to recapitulate bTB disease, highlighting Δ*phoPR* as a potential vaccine candidate. We have established a system for measuring behaviours indicative of dissemination to neuronal sites in the C3HeB/FeJ model and have utilised recombineering to make mutants in candidate genes involved in pathogen in vivo survival in order to assess dissemination.

Disease outcomes are also influenced by heterogeneity in the host response independent of pathogen genetics. We have observed that C3HeB/FeJ shows a heterogenous response to infection with AF2122/97 with dissemination being associated with unrestrained neutrophil influx. Using in *vitro models* of disease states, we have shown that Mb is able to survive under conditions associated with sub-clinical disease (hypoxia) remodelling its proteome to enable persistence and identified the pathogen genes required to survive immune stress (oxidative onslaught).

### PERSPECTIVES AND RECOMMENDATIONS FOR FUTURE ACTIVITIES

It will be important to see how genetic diversity in circulating strains of Mb influence disease progression to determine the field relevance of knowledge derived from Mb AF2122/97. Future research will aim to identify additional Mb genes essential for survival and dissemination in vivo. Refinement of animal models, such as the C3HeB/FeJ mouse, will enhance our understanding of disease progression. Understanding the heterogeneous host immune response, particularly the role of neutrophils, may inform the development of host biomarkers to predict infection outcomes and hence improve intervention strategies. Understanding differences between Mtb and Mb in persistent states will inform species specific adaptation to preferred hosts.

# ScResGoats



## Classical scrapie in genetically resistant goats: questioning current concepts and policies

**COORDINATOR:** Dr John Spiropoulos, Pathology department, APHA, Weybridge, United Kingdom
**PARTNERS:** ELGO-DIMITRA, VRI, Thessaloniki, Greece | IZSPLV, Turin, Italy | CISA-INIA-CSIC, Madrid, Spain
**PROJECT PERIOD:** April 2023 – March 2026

### HIGHLIGHTS/MAJOR ACHIEVEMENTS

Classical Scrapie (CS) is a fatal neurodegenerative disease affecting sheep and goats. It is caused by pathogenic proteins ($PrPS^c$), called prions, which are abnormal forms of a naturally occurring protein ($PrP^C$). In sheep the only effective method to eradicate CS is to implement breeding for resistance to the disease (BRD) schemes taking advantage of $PrP^C$ polymorphisms which confer genetic resistance (GR) to CS. In goats, a GR polymorphism at codon 222 has been identified in most breeds, although its prevalence is low, providing hope that BRD schemes can be implemented in this species too.

In this project we are studying the properties of CS cases from Greece that have been detected in GR animals at codon 222 to identify potential factors that may interfere with BRD.

We have identified all existing GR cases from Greece and collected any available materials. No biochemical differences were identified between GR and genetically sensitive (GS) cases. Interestingly, it was shown that the molecular profile $PrPS^c$ from goats from Greece is different compared to the rest of goats from EU irrespective of genotype at codon 222. Proliferation of prions from GR cases in vitro using various $PrP^C$ substrates (Figure 1), showed that GR cases proliferate with ease to GS ovine backgrounds. Interestingly, the ovine GR substrate did not inhibit proliferation of prions that derived either from GR or GS sources, albeit with limited success, irrespective of $PrP^C$ genotype. The zoonotic potential of GR and GS sources was tested by assessing their proliferation in human $PrP^C$ backgrounds. Both sources failed to transmit in a human substrate that is associated with susceptibility to variant Creutzfeldt-Jakob (vCJD), a human disease which is linked to BSE. Although no GR cases proliferated on the human background that confers resistance to vCJD some GS cases did transmit.

### PERSPECTIVES AND RECOMMENDATIONS FOR FUTURE ACTIVITIES

There may be strain differences between geographical regions which can affect the success of BRD schemes.

GR backgrounds in goats are not as watertight as previously thought and further research is required to identify any implications their introduction might have.

CS strains that are proliferating in GS goats may have zoonotic potential.

## *IN VITRO* PROLIFERATION OF GR AND GS CLASSICAL SCRAPIE CASES IN VARIOUS SUBSTRATES

| | $PrP^C$ of classical scrapie cases | |
|---|---|---|
| $PrP^C$ of substrate | GR | GS |
| Ovine GS | Yes | Yes |
| Ovine GR | Restricted | Minimal |
| Bovine | Minimal | Ongoing |
| Human - A | No | No |
| Human - B | No | Restricted |

**GR** is associated with genetic resistance to classical scrapie
**GS** is associated with genetic susceptibility to classical scrapie
**Human - A** substrate is associated with genetic susceptibility to variant Creutzfeldt-Jakobs disease (vC-Jd)
**Human - B** substrate is associated with genetic resistance to vC-Jd

# SciCe

## Classical Scrapie in Iceland, a model for prion diseases worldwide

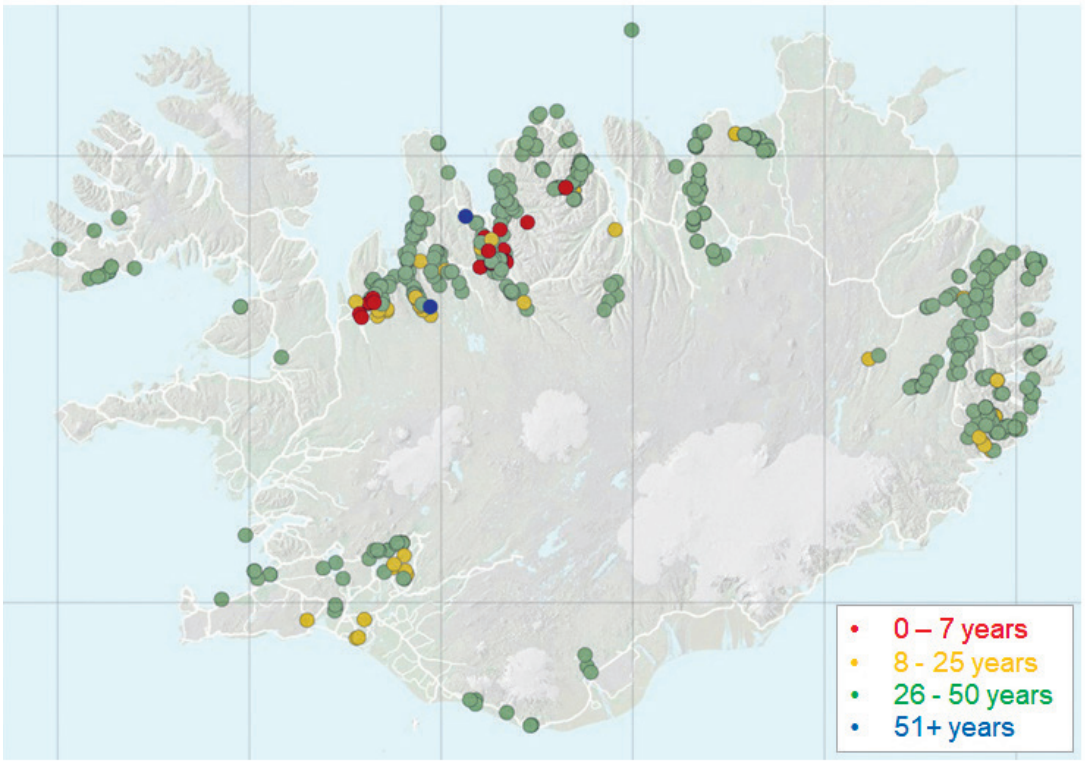
**COORDINATOR:** Dr. Christine Fast, FLI-Isle of Riems, Germany
**PARTNERS**: APHA, United Kingdom | Justus Liebig University of Gießen, Germany | The Roslin Institute, University of Edinburgh, United Kingdom | INRAE, France | CISA-INIA, Spain | ISS, Italy | University of Iceland at Keldur, Iceland
**PROJECT WEBSITE**: https://www.fli.de/en/institute/institut-fuer-neue-und-neuartige-tierseuchenerreger-innt/projekte/research-projects-single-view/scrapie-in-island-a-model-for-prion-diseases-worldwide-scice/
**PROJECT PERIOD:** April 2023 – March 2026

### HIGHLIGHTS/MAJOR ACHIEVEMENTS

Prion diseases are caused by the pathological prion protein (PrPSc), which is found in a diversity of prion strains (PS) that exhibit specific biological behaviours. The mechanisms controlling PS evolution remain unclear. In classical scrapie (CS), polymorphisms of the prion protein gene affect susceptibility to PS and influence the transmission and evolution of PS. Potentially protective alleles have been detected in Icelandic/Greenlandic sheep, and preliminary data from case-control studies in Iceland/EU showed that no such sheep was CS-positive. These alleles show in vitro that they are less convertible than the wild type and transgenic mice were generated to test the protective potential in vivo. A selection of Icelandic CS isolates showed different biochemical properties and variable conversion efficiencies in vitro, indicating diverse PS in Iceland. A further PS characterization by mouse bioassay is ongoing. Preliminary results indicate persistent environmental reservoirs of CS in Iceland, further analysis along with epidemiological data may even enable the examination of PrPSc adaptation. A survey of CS affected and unaffected farms in representative Icelandic regions (Figure 1) identified risk factors and revealed various weaknesses in control/eradication measures. The development of an economic model for CS response is ongoing.

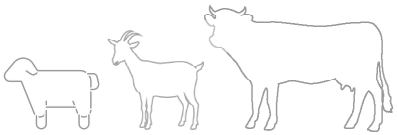
### PERSPECTIVES AND RECOMMENDATIONS FOR FUTURE ACTIVITIES

Breeding for CS resistance is already underway to eradicate CS in Iceland and changes have also been made to control measures, but their effectiveness needs to be monitored, particularly in relation to the risk factors identified, including the environmental reservoirs. Nevertheless, it can be expected that the culling of scrapie-affected herds will be reduced, thereby lowering the cost of disease control while maintaining productivity and animal welfare. The identified polymorphisms will allow greater flexibility for breeding strategies worldwide, maintaining diversity and specific production traits around the globe. Furthermore, the results already indicate that the diversity of PS in Iceland differs from that in the EU, but analysis of the mouse bioassays is still needed to draw conclusions about the occurrence of PS and their zoonotic potential. The data gained here, using CS as a model, will be critical for controlling or preventing re-emergence of known diseases or emergence of new PS which might carry zoonotic threats.




Icelandic classical scrapie outbreaks per year and region with a main cluster of new outbreaks in the North Western part of Iceland. All cluster regions are represented in the selection of isolates and in the epidemiological analysis.

# Q-Net-Assess



## Improved molecular surveillance and assessment of host adaptation and virulence of Coxiella burnetii in Europe

**COORDINATOR:** Professor Tom McNeilly, Moredun Research Institute, United Kingdom
**PARTNERS**: Moredun Research Institute, United Kingdom | FLI, Germany | Sciensano, Belgium | Royal GD, The Netherlands | NEIKER, Spain | ANSES, France | INRAE, France
**PROJECT WEBSITE:** https://q-net-assess.com
**PROJECT PERIOD:** April 2023 – March 2026

### HIGHLIGHTS/MAJOR ACHIEVEMENTS

The overall aim of this project was to standardise sample collection from *C. burnetii* cases, develop improved methods to isolate the bacteria from different clinical samples, and sequence and strains in a coordinated manner such that the genetic determinants of *C. burnetii* host adaptation and pathogenicity can be determined.

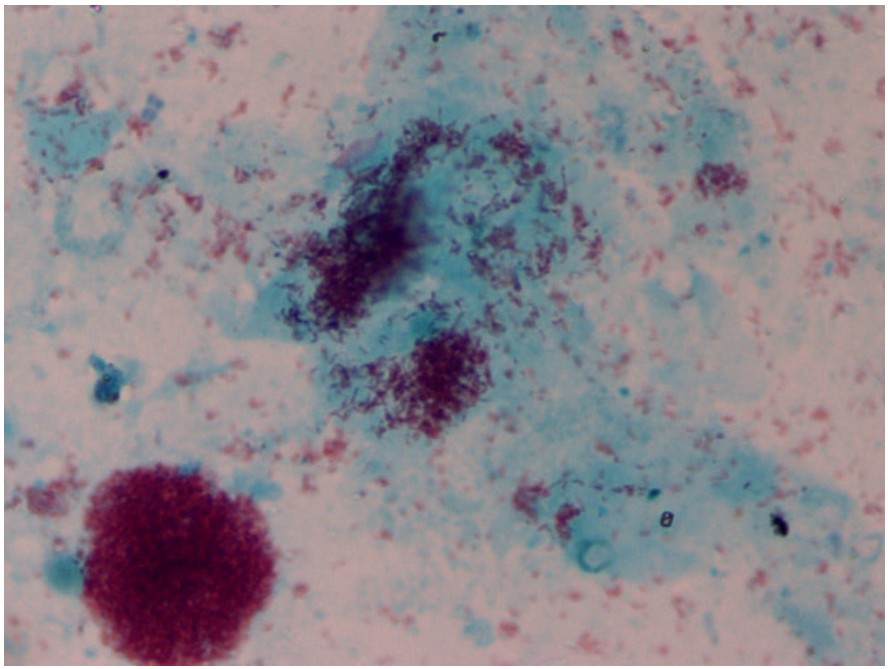
Important achievements to date are:

- Establishment of a strong partnership between European Q fever reference laboratories and researchers. This includes the addition of eight hop-on partners to the project, including national reference laboratories from Spain, Austria and Italy (check others) and has allowed the standardisation of isolation and genomic surveillance methodologies across Europe.
- Development of improved protocols for isolation of *C. burnetii* from clinical samples, including optimisation of axenic media formulations to allow simplified propagation of the bacteria *in vitro.*
- Development of *in vitro* phenotypic assays for characterisation of *C. burnetii* strain virulence.
- Successful development of direct sequencing approaches to obtain *C. burnetii* genomic data directly from clinical samples. This is potentially 'game-changing' as genetic information can now be obtained without the need for isolation of C. burnetii at BSL3. Also, this approach allows genomic information of other abortifacient agents present within the sample.
- Generation of the most comprehensive whole genome *C. burnetii* phylogeny to date, which includes strains isolated from different host species across Europe.

### PERSPECTIVES AND RECOMMENDATIONS FOR FUTURE ACTIVITIES

An important legacy of this project is to establish routine isolation and/or sequencing of *C. burnetii* by surveillance laboratories across Europe, such that the genomic database for *C. burnetii* is continually expanding and improving. We are creating a database hosted by ENA where *C. burnetii* genomic data and associated metadata will be stored. Importantly, guidance will be provided on the minimum requirements for data upload to ensure the future quality and usefulness of the database.

Having established a strong European network of laboratories and researchers with expertise in livestock abortions and zoonoses, we recommend that the network continues to function. Building on the promising results with direct sequencing approaches, we recommend that the network expands its research to consider other abortifacient agents where genomic surveillance is currently lacking or absent.



Coxiella burnetii organisms (red) in a placental smear from a case of Q fever abortion in a goat. © Crown copyright 2023. Licensed under the Open Government Licence v3.0.

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# FLU-SWITCH

## Identification of factors driving the emergence and spread of avian influenza viruses with zoonotic potential

**COORDINATOR:** Romain Volmer, Ecole nationale vétérinaire de Toulouse, Université de Toulouse, ENVT, INRAE, IHAP, UMR 1225, Toulouse, France

**PARTNERS:** FLI, Insel Riems, Germany | Utrecht University, The Netherlands | EU/OIE/National Reference Laboratory for Avian Influenza and Newcastle Disease | FAO Reference Centre for Animal Influenza and Newcastle Disease | Istituto Zooprofilattico Sperimentale delle Venezie, Italy | The Roslin Institute, University of Edinburgh, United Kingdom | APHA-Weybridge, United Kingdom | University of Warsaw, Poland | Izmir Biomedicine and Genome Center, Izmir, Turkey

**PROJECT PERIOD:** October 2023 – September 2026

### HIGHLIGHTS/MAJOR ACHIEVEMENTS

The FLU-SWITCH project addresses the zoonotic potential associated with the switch of H5 and H7 low pathogenicity avian influenza viruses (LPAIV) to high pathogenicity avian influenza viruses (HPAIV). Evolution of the typical LPAIV monobasic HA cleavage site (CS) to a multibasic CS is critical to produce an HPAIV and is associated with an increased zoonotic potential.

FLU-SWITCH has allowed major progress on the identification of the receptor binding properties of HPAIV. These include the demonstration that specificity of HA binding to sialic acid receptors depends on the clade of H5Nx HPAIV and the identification of neuraminidase as a modulator of sialic acid receptors binding specificity. Significant progress has also been made on the understanding of evolutionary and epidemiological dynamics of HPAI H5Nx viruses in Europe (2020–2022) and on the collection of samples, which will allow to study HPAIV evolution under vaccination pressure. Finally, FLU-SWITCH partners have launched FluMut, an innovative tool that enables the rapid analysis of viral sequences to identify key zoonotic molecular markers.

### PERSPECTIVES AND RECOMMENDATIONS FOR FUTURE ACTIVITIES

FLU-SWITCH has also generated a number of tools and preliminary results that should shed new light on the virological and host factors modulating the emergence of HPAIV. In particular, significant progress has been made to understand whether all LPAIV of the H5 and H7 subtype have the same risk of evolving to HPAIV, or whether specific genetic features in the HA or other genes are associated with a higher risk of LPAIV to HPAIV switch. FLU-SWITCH will also investigate how host factors modulate HPAIV emergence, either by influencing the risk of acquisition of a multibasic CS, or the control of newly emerged variants by the innate immune response. FLU-SWITCH is also developing AIRA, a risk assessment tool designed to evaluate the likelihood of LPAIV-to-HPAIV transition by integrating data from the literature, from public databases and the outputs of the FLU-SWITCH project.

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# NanoZoo

## Protein nanoparticle vaccine platform for rapid response against zoonotic viruses in poultry and swine

**COORDINATOR:** Gorben Pijlman, Wageningen University, the Netherlands
**PARTNERS**: Wageningen University (WU), Belgium | Oxford Brookes University (OBU), United Kingdom | University of Copenhagen Denmark (UC), Denmark | QIMR Berghofer Medical Research Institute (QIMRB), Australia | MSD Animal Health Netherlands (MSD-AH), The Netherlands | AdaptVac (AV), Denmark
**PROJECT WEBSITE:** https://www.icrad.eu/portfolio-items/nanozoo
**PROJECT PERIOD:** November 2023 – June 2025

### HIGHLIGHTS/MAJOR ACHIEVEMENTS

Novel vaccine platforms are urgently needed to produce efficacious, safe, low cost, and rapidly adaptable ('plug-and-play') vaccines to face the threat of zoonotic viral diseases in livestock. Protein nanoparticle vaccines, e.g. the highly successful porcine circovirus and the human papillomavirus vaccines, are generally considered an optimal vaccine format because of their high efficacy and intrinsic safety. Built on our success with clinical testing of a nanoparticle vaccine against covid-19 (H2020 Prevent-nCoV, clinical trial COUGH-1), the aim of the NanoZoo project is to apply this unique protein nanoparticle vaccine platform for rapid response against zoonotic viruses in poultry and swine. This technology involves expression of viral antigens in insect cells combined with antigen presentation on protein nanoparticles to induce a superior immune response. In the NanoZoo project, the two-component nanoparticle vaccine platform is applied for developing novel vaccines against important zoonotic viral diseases in poultry, and emerging vector-borne zoonotic viral diseases in swine. The project brings together academic and industry experts in viral antigen expression, nanoparticle vaccines and animal health.

Viral glycoproteins and immunodominant protein domains of the target viruses are expressed in insect cells using the robust baculovirus expression system to ensure correct folding and glycosylation of the antigen (WP1). Optimization of the antigen design for the vaccine targets is ongoing at WU and MSD-AH.

The production process was scaled-up at OET/OBU and optimized to ensure efficient protein production with high quality and purity (WP1). The viral antigens were shown at AV/UC to be coupled onto self-adjuvanting protein nanoparticles, and the coupling conditions are currently being optimized to maintain proper nanoparticle stability (WP3).

Moreover, the baculovirus expression system was engineered as a very fast 'plug-and-play' platform by OET/OBU to go in a single step from a synthetic gene to viral antigen production (WP2), which will outcompete novel mRNA vaccine platform technologies in terms of speed, volume and cost.

### PERSPECTIVES AND RECOMMENDATIONS FOR FUTURE ACTIVITIES

Vaccine candidates for Japanese Encephalitis virus and Getah virus are now ready for further evaluation in vaccination studies (with/without adjuvants) in relevant animal models at partner QIMRB (WP4).

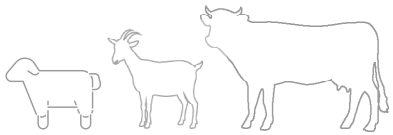
Antigens for Newcastle disease virus and other pathogen targets are in the design phase at WU and MSD-AH. Expression data at OET/OBU will be generated to continue with antigen formulation at AV/UC.

**CALL 3:**

# Helminth infections and changing climate: tackling the challenges for animal health

The overall objective of this research call is to increase understanding and preparedness for impending effects on animal health and the livestock industry caused by climate change and spread of anthelmintic resistance. This includes basic research to better understand mechanisms behind these topics or applied development of detection, management, intervention and prevention strategies.

# ANTHELMOGRAM



## The next generation decision making tool for anthelmintic resistance management in Europe

**COORDINATOR**: Dr. Cedric Neveu, INRAe, UMR 1282 ISP, France
**PARTNERS**: University of Glasgow, United Kingdom | Ghent University, Belgium | Kreavet, Belgium | INVENesis France Sàrl, France | Faculty of Veterinary Medicine, Afyon Kocatepe University, Turkey | Micron Agritech, Ireland
**PROJECT PERIOD:** January 2025 – December 2027

### HIGHLIGHTS/MAJOR ACHIEVEMENTS

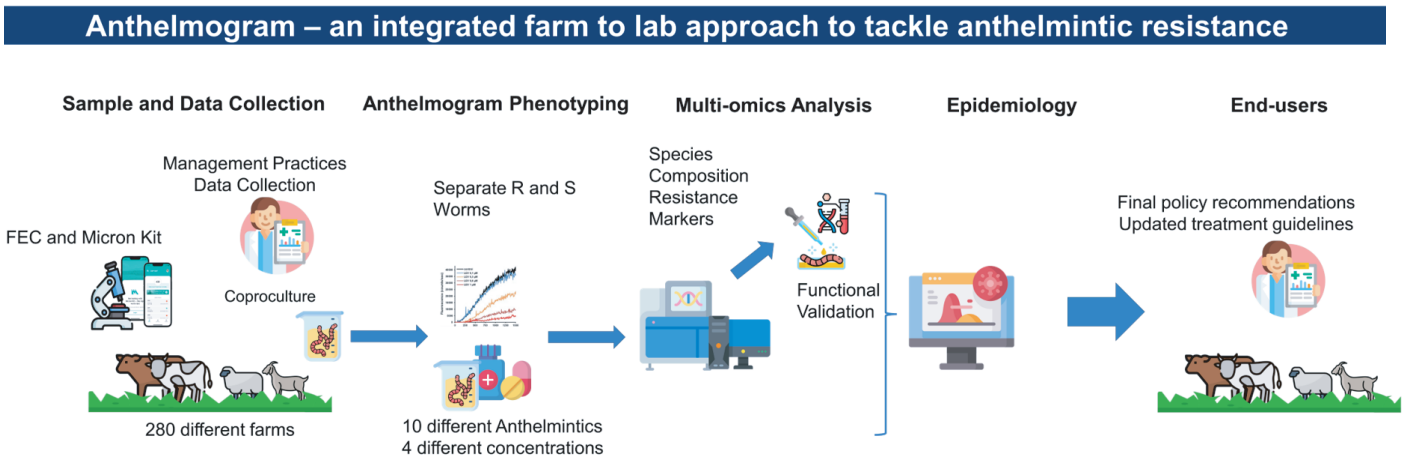
Mirroring the principle of an "antibiogram" test, the Anthelmogram project aims to provide a unique decision tool addressing the limitations of current Anthelmintic Resistance (AR) diagnostics. Taking advantage of a newly developed high throughput automated larval motility assay, the Anthelmogram platform can phenotype up to 4000 samples per week and accurately determine the resistance/susceptibility status to 10 different anthelmintic compounds per parasite population. The Anthelmogram assay will be applied to parasitic nematodes of cattle, sheep and goats from 6 distinct countries representing key biogeographical ruminant farming regions of Europe and Anatolia where AR is a major concern.

The Anthelmogram consortium gathers European leaders in the field of AR research and will fully utilise the unique set of biological material and data generated by the platform to perform unparalleled epidemiological and molecular studies on parasitic helminths.

The expected outcomes include: **1)** Large scale sampling and systematic automated phenotyping of worms sampled in 280 farms; **2)** Creation of a biobank of resistant/susceptible helminth populations for use by the research community; **3)** Generation of novel data on the epidemiology of drug resistance and susceptibility in ICRAD countries; **4)** Large scale genetic characterisation of phenotyped helminth populations for the discovery and functional validation of AR molecular markers; **5)** Evaluation of the efficacy of "sustainable approaches" to the management of resistance using longitudinal data collected during the project; **6)** Concrete policy recommendations and updated best practice guidelines for AR management in Europe.

### PERSPECTIVES AND RECOMMENDATIONS FOR FUTURE ACTIVITIES

Whereas antimicrobial resistance management benefits from the critical input of the "antibiogram test", the lack of an equivalent assay for helminths represents a major bottleneck for the rational use of anthelmintic in the field and an important limitation for both epidemiological and molecular research. Anthelmogram seeks to address this lack/gap by developing a cutting-edge technology to diagnose, prevent, and manage AR. In addition to providing a unique decision-making tool, the large-scale sampling coupled with an accurate phenotyping assay will pave the way for unparalleled research into molecular mechanisms of resistance, evaluation of control strategies and spatial mapping of resistance. Therefore, Anthelmogram will constitute a unique multidisciplinary research pipeline ensuring European preparedness to control AR.


Anthelmogram – an integrated farm to lab approach to tackle anthelmintic resistance
Sample and Data Collection
Anthelmogram Phenotyping
Multi-omics Analysis
Epidemiology
End-users
Management Practices
Data Collection
FEC and Micron Kit
Coproculture
280 different farms
Separate R and S
Worms
10 different Anthelmintics
4 different concentrations
Species
Composition
Resistance
Markers
Functional
Validation
Final policy recommendations
Updated treatment guidelines

# METABOL-AR

## An application of metabolomics to the detection of anthelmintic resistance of gastrointestinal nematodes to benzimidazoles in goats

**COORDINATOR:** Dr Marcin Mickiewicz, Division of Veterinary Epidemiology and Economics, Institute of Veterinary Medicine, Warsaw University of Life Sciences, Poland
**PARTNERS:** Warsaw University of Life Sciences, Poland | Latvia University of Life Science and Technologies, Latvia | French Agency for Food, Environmental and Occupational Health & Safety, France | Queen's University Belfast, United Kingdom | Veterinary School of Alfort, France | Istanbul University-Cerrahpasa, Turkey | Estonian University of Life Sciences, Estonia
**PROJECT WEBSITE:** https://www.icrad.eu/portfolio-items/metabol-ar
**PROJECT PERIOD:** April 2025 – March 2028

### PROJECT OBJECTIVES

- Determination of the influence of geographical and climatic factors on larvae metabolism.
- Identification of trace differences in metabolomic profile associated with variations in gastrointestinal nematode species composition.
- Identification of a set of metabolites accurately distinguishing between benzimidazole-susceptible and benzimidazole-resistant larvae.
- Obtaining proof-of-concept for metabolomics as a rapid and practical test for resistance to benzimidazoles.
- Spread of know-how, implementation of new methods and provision of training in anthelmintic resistance diagnostic methods in countries where they have not been used before.
- Collection of data on prevalence of benzimidazole resistance in goat herds from areas where they have not been available until now (Estonia and Latvia) or are scarce (Turkey).
- Collection of detailed data on the species and community composition in goats in different climatic regions of Europe.

### PERSPECTIVES AND RECOMMENDATIONS FOR FUTURE ACTIVITIES

The project results may be used to expand research on resistance to other anthelmintics (AHs) in which the genetic basis of resistance is not as well-known as in benzimidazole (BZs). Thus, successive anthelmintic drug groups have had a shorter effective life, and this may indicate genetic selection and upregulation of common general chemical defence mechanisms. The presence of specific metabolites and their comparison to the metabolomic profile of BZ susceptible larvae may help identify previously unknown genes that could be responsible for resistance to other AHs. Additionally, the project aims to provide data on the metabolic adaptation of larvae to specific climatic conditions. This may indicate the direction of further genetic research on the expression of genes responsible for specific metabolic pathways related to larvae survival in the environment.

METABOL-AR

# MAP-TCBZR

## Mapping resistance loci and interrogating mechanisms of triclabendazole resistance in European isolates of Fasciola hepatica

**COORDINATOR:** Prof Jane Hodgkinson, Institute of Infection, Veterinary and Ecological Sciences, University of Liverpool, United Kingdom
**PARTNERS:** University College Dublin, Ireland | Queen's University Belfast, Northern Ireland, United Kingdom | Kreavet, Belgium | ARSIA, Belgium | Van Yüzüncü Yıl University, Turkey
**PROJECT PERIOD:** October 2024 – September 2027

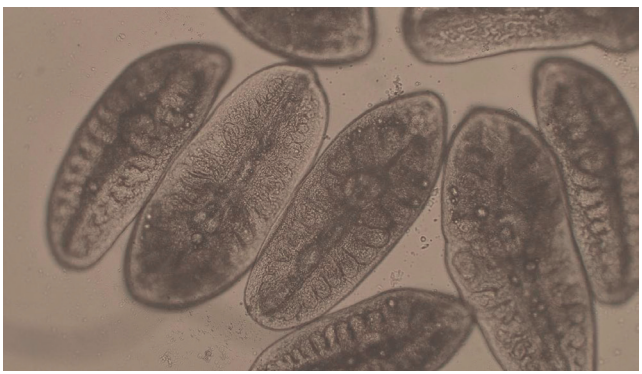
### PROJECT OBJECTIVES

The project aims to improve detection of anthelmintic resistance in liver fluke, Fasciola hepatica, and mitigate the impact drug resistance has on ruminant health and production. F. hepatica is one of the most intractable parasitic infections affecting farmed ruminants in Europe, causing widespread production losses, disease and mortalities. Resistance to flukicide anthelmintics means an inability to effectively control liver fluke infection, exacerbating the difficulties experienced by livestock producers. Triclabendazole (TCBZ) is unique in its ability to kill early immature liver flukes that cause acute disease and mortality, especially in sheep. Resistance to TCBZ is hugely problematic, resulting in substantial economic loss and impacting negatively on livestock health and welfare.

Accurate diagnosis of TCBZ resistance allows farmers to make informed decisions about fluke control, such as using alternative, effective flukicides against adult stages earlier in the transmission cycle. The project builds upon surveillance expertise developed within each country to take a coordinated and standardised approach to map the location of flukicide resistance in sheep flocks in high fluke infection areas within Europe. By taking a harmonised multi-farm, multi-country approach, this project constitutes the largest study of the drivers for, and detection of, TCBZ resistance worldwide. This project will integrate both forward and reverse genetics approaches to develop a functional understanding of TCBZ resistance. Mapping genomic signatures of drug selection in a range of isolates from different locations will address whether TCBZ resistance has a common underlying mechanism, whilst our parasite growth platform will allow us to explore the role of candidate genes pinpointed by our genomic studies. The deliverables will represent a step-change in knowledge.

### PERSPECTIVES AND RECOMMENDATIONS FOR FUTURE ACTIVITIES

Our outputs will raise awareness of drug resistance on farm, facilitate the development of new diagnostics to accurately predict drug resistance at scale and foster new collaborations across Europe, for both early career and established researchers. Our work will allow farmers to plan the strategic use of flukicides, vets and prescribers to better advise their clients, policymakers to consider regulatory changes, and industry to evaluate new and existing drugs.



Juvenile liver fluke grown in vitro.

# HARTEMIS

## Haemonchus Anthelmintic ResisTance - Evolution Mechanisms and Innovative Solutions

**COORDINATOR:** Anne LESPINE, Toulouse University INTHERES, INRAE, France
**PARTNERS:** IHAP, Toulouse Veterinary School, France | Queen's Univ. Belfast, United Kingdom | Warsaw Univ. of Life Sciences, SGGW, Poland
**PROJECT PERIOD:** January 2025 – December 2027

### PROJECT OBJECTIVES

Gastrointestinal nematodes (GIN) represent a major threat to animal welfare, health, and productivity in small ruminant farming. Anthelmintic drugs remain essential to control these parasites, particularly macrocyclic lactones (MLs), which are the cornerstone of current treatment protocols. However, the widespread and often unsustainable use of these drugs has led to increasing levels of anthelmintic resistance (AHR), jeopardizing parasite control across Europe. Among GIN species, Haemonchus contortus is particularly problematic due to its high pathogenicity, prolific reproductive potential, genetic variability, and its ability to benefit from rising temperatures under climate change scenarios. This combination of biological traits and environmental drivers makes it a key model for studying parasite adaptation and resistance.

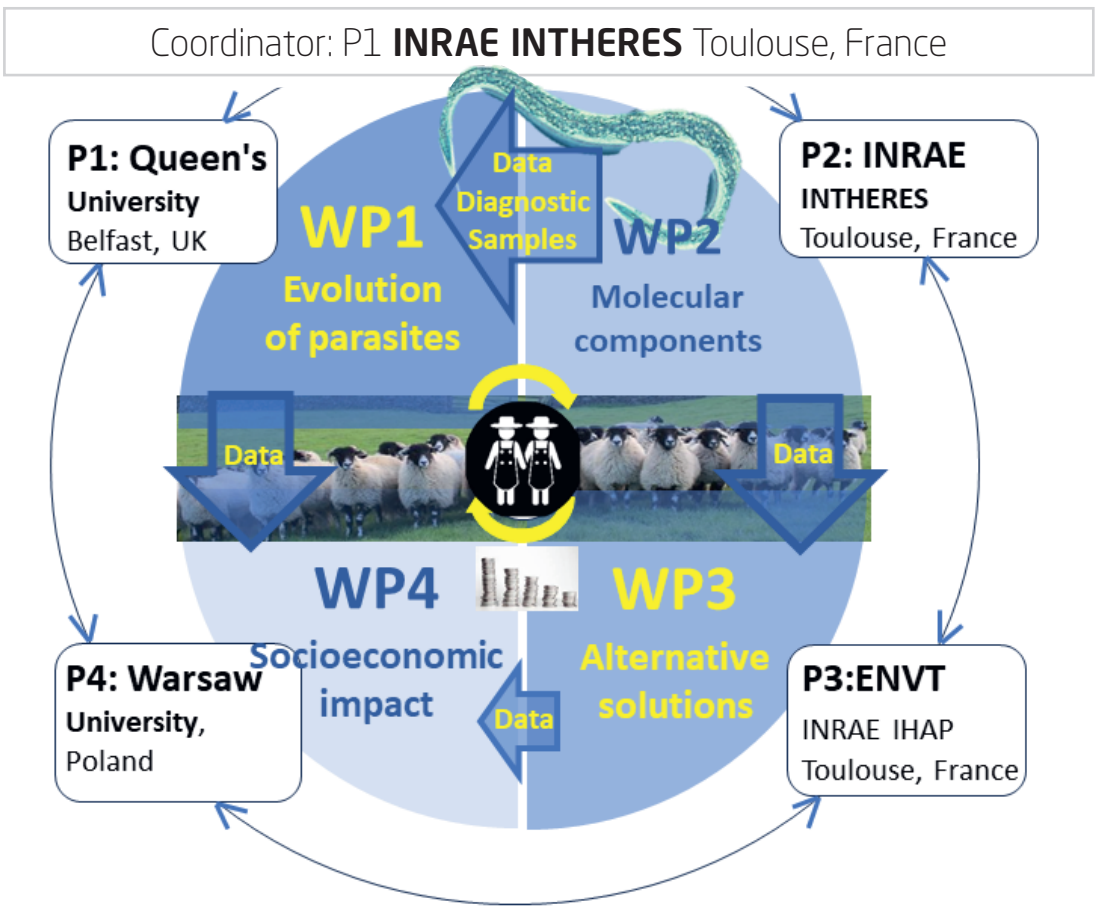
This project aims to address these challenges through four interconnected objectives

- Track the evolution of GIN populations and AHR by collecting and characterizing resistant and susceptible H. contortus isolates from sheep farms in three climatically diverse European regions.
- Investigate the molecular and physiological mechanisms underlying drug resistance and temperature adaptation using C. elegans and H. contortus models, and identify potential molecules with improved efficacy.
- Translate scientific findings into actionable, regionally adapted strategies for farm-level parasite management, aiming to reduce infection pressure and delay resistance development.
- Evaluate the economic benefits of these interventions, promoting feasible and cost-effective approaches for sustainable parasite control.

By integrating field epidemiology, fundamental research, and practical applications, this project seeks to support more resilient, adaptive control strategies in the face of both drug resistance and climate change.

### PERSPECTIVES AND RECOMMENDATIONS FOR FUTURE ACTIVITIES

- Deepen mechanistic understanding of AHR to inform diagnostics and treatment choices.
- Propose realistic, evidence-based strategies to mitigate AHR in sheep production systems.
- Develop predictive tools to anticipate parasite population shifts and resistance trends.
- Incorporate climate change projections to design long-term, sustainable parasite control programs adapted to future environmental conditions.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P3:ENVT INRAE IHAP Toulouse, France
P4: Warsaw University, Poland
WP1 Evolution of parasites
WP2 Molecular components
WP3 Alternative solutions
WP4 Socioeconomic impact
Data Diagnostic Samples
Data
Data
Data

# Funding organizations

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