Animal Health Research Centre (CISA)
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The Centre for research on Animal Health, located in Valdeolmos, Madrid, is a centre for research and surveillance in prevention, diagnosis and control of animal transmissible diseases. It is particularly focused on emerging, re-emerging and transboundary infectious diseases of livestock causing economical and sanitary impact, including zoonosis that may cause restrictions in the international trade and a serious effect on public health and food security. Its primary mission is to promote advanced research, technology development, cooperation with national and international bodies, and technology transfer in the area of animal health, following the strategic priorities agreed by national and international forums and organizations of relevance in the area.

CISA Biosafety (BSL) facility is nearly 10.824 m² in extension, and it is made up by 40 BSL3 laboratories (14 in common use) where specialized studies and technical assistance are performed under the highest quality standards for the continuous improvement of prevention and control of diseases. Restricted laboratories for research studies on Prions and FMD are in the site. Two BSL4 (OIE) Laboratories are designed to work with infectious agents that could affect humans, by using special suits that ensure the isolation and security of staff. Outside of the BSL facility, a restricted BSL-2 Area is located, consisting of 10 laboratories equipped with the appropriate infrastructure for epidemiology and modelling, environmental health, sequencing and molecular biology studies of infectious diseases, including wild life species.

The CISA Biosafety facility belongs to the Network of High Biosafety Laboratories (RLASB) in the map of Singular Scientific Technical Facilities (ICTS) of MINECO. Also, It takes part of the Biological Alert Laboratory Network (RE-LAB), under the Presidency of the Government and dedicated to dealing with threats caused by biological agents. In addition to the active collaboration as a partner in research projects with many European institutes and others worldwide, CISA actively participates in a number of networks of excellence, emphasizing EPIZONE, DISCONTOOLS, VetBioNet, and Global Platforms for Epizootic Diseases, diagnosis and control. The MEDILABSECURE animal virology area of a “One health” laboratory network is coordinated by CISA to set up a liaison with nineteen countries around the Mediterranean and Black Sea, for capacity building to face alerts caused by zoonotic viral threats.

INIA-CISA is Reference Laboratory for EU and for the Food and Agriculture Organization of the United Nations (FAO) for African Swine Fever (ASF). This nomination requires of great capacity to respond to emergencies and outbreaks associated to this disease. As such these laboratories develop an intense activity in diagnostic matters, provision of standards and controls, and harmonization of National Reference Laboratories. An active regular collaboration is established with more than fifty countries worldwide.

INIA-CISA is Reference Centre in Biosafety for FAO (INIA-CISA) carrying out advising on biosafety matters and bio-contention facilities.

There is an strong collaboration with the Ministry of Agriculture of Spain supporting to National reference laboratories in their needs and other scientific-technical matters affecting livestock.

RESEARCH GROUPS

- Epidemiology and Environmental Health
- Emerging and Transboundary Diseases
- New Strategies for the Control of relevant Pathogens in Animal Health
- Fish Immunology and Pathology
- Molecular and Cellular Biology of Prions
- Immunoprofilaxis of Vector-Borne Viral Diseases

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Our principles are focused on the ONE HEALTH paradigm, assessing the livestock-wildlife-environment interphase. Main research lines:

Spatio-temporal epidemiology of transboundary and emerging infectious diseases of livestock. Its purpose is to diagnosis the epidemics (prevalence, transmission rate and speed, role of the hosts...) developing exploratory tools and to identify risk factors and/or protective measures that can condition the magnitude of the epidemic and its temporal and territorial distribution. An important part consists in the modelling of potential distribution of wild species. Main goals during 2016-2017 are the risk assessment of African Swine Fever (ASF) introduction in the EU through wild boar, the development of a homogeneous database on ASF notifications and a cartographic tool on wild boar distribution in Eurasia.

Diagnosis and epidemiology of main zoonotic and/or economic relevant diseases for livestock and the environment. It is addressed through an epi-lab approach based on control strategies evaluation, cost-benefit analysis, higher sensitive diagnostic techniques development, genotypic characterization of isolates and molecular epidemiology. During 2016-2017, we have been focusing on bovine viral diarrhoea virus (BVDV) to identify the types and subtypes present in the Spanish productive systems and to study the evolution of viral subpopulations. We have also assessed the dynamics and viability of hives against the colony collapse syndrome of bees through the characterization of the Mediterranean scenario (weather, landscape, colony and management) and the identification of suitable areas.

Surveillance of antimicrobial resistance. Alternative methods for direct, multiplex and sensitive detection and quantification of antimicrobial resistance genes (ARG) in the resistome have been developed. Since the vast majority of the microbiota is not cultivable and most ARGs of epidemiological relevance are plasmid-mediated, this culture-independent method is highly encouraged for environmental studies, but also livestock and wildlife. It has been applied in 2016-2017 on national and international studies, e.g. composting effect on ARGs reduction and; livestock impact across a river basin in Costa Rica. On the other hand, we conduct environmental risk assessment on veterinary medicines linked to marketing authorizations.
Our field of work is R&D&i in emerging and transboundary diseases representing serious threats to animal health, such as: African swine fever (ASF), classical swine fever (CSF), swine vesicular disease (SVD), foot-and-mouth disease (FMD), bluetongue (BT), peste-des-petits ruminants (PPR), Rift Valley fever (RVF), Crimea-Congo haemorrhagic fever (CCHF), and West Nile fever (WN), among others. The team is in charge of the European Union and FAO reference laboratories for ASF, and gives scientific and technical assistance to National Reference Laboratories (NRL) of Spain added to a strong commitment to international cooperation.

With regards to ASF, activities are mainly focused on biological and molecular characterization studies of ASF virus isolates currently affecting European countries, including massive sequencing. The main goal is to obtain a precise description of the genetic, clinical, virological and pathological features induced in animals infected with currently circulating ASFV isolates.

Second major research line is focused on WN, encompassing studies on pathogenesis, pathogen-host interaction, and cross-protection of different flaviviruses in mammals and in a range of avian natural host species, added to molecular characterization of recent Spanish WNV isolates to follow up the spread and evolution of the virus.

Finally, our group is significantly involved in the development, updating and transfer of diagnostic methods for relevant infectious animal diseases such as ASF, WN and other flaviviruses, PPR, RVF, AI, BT or CCHF.

As EU and FAO ASF reference laboratory, an extensive work on scientific advising and scientific-technical assistance is carried out all over the world. Our actions comprise missions, workshops, meetings, and training activities for international organisms and institutions (EFSA, SANTE (EC), FAO Regions), and for the veterinary services and animal health institutes of affected countries or those at risk of ASF. Throughout these activities, international cooperation is currently established with more than 40 countries.

The team is also coordinating the animal virology area, through the MediLab-Secure project, of a “One health” laboratory network covering 19 non-EU countries around the Mediterranean and Black Sea regions, organising a number of activities for capacity building to face alerts caused by zoonotic viral threats.


New Strategies for the Control of Relevant Pathogens in Animal Health

The main aim of our group is to promote Animal Health by the prevention of animal diseases by developing new strategies for vaccine improvement against important pathogens in Animal Health. Control strategies for most of the diseases in Animal Health are based on killed or attenuated vaccines of limited effectiveness and with serious risks. This indicates the need of developing new vaccine strategies that incorporate the potential of technological innovation. Thus, we are working on i) generation of Calicivirus VLPs as a platform for antigen presentation, ii) generation of recombinant non-replicative adenovirus as a new vaccine design, iii) analyses of immune responses against viral infections in natural hosts, and iv) identification and characterization of viral epitopes.

In the 2016-2017 period, we have described a novel mechanism of viral induced-immunosuppression by bluetongue virus (BTV) during infection in sheep, in which disruption of follicular dendritic cells by BTV hinders B-cell division. This results in delayed production of high affinity and virus neutralizing antibodies very early after infection, delaying host immune response that likely affects virus systemic dissemination and the clinical outcome of the disease. The study of T cell responses during BTV infection in sheep showed that CD4+ and CD8+ T cell populations expanded whereas recall responses to BTV challenge led to BTV-specific expansion and activation of CD8+ but not CD4+ T cells. Moreover, striking differences in repertoire development were noted over the time-course of infection. We have also studied the immune response elicited by peste des petits ruminants virus (PPRV), describing CD4+ and CD8+ T cell epitopes in the F and H proteins. As a new approach to boost the immune response against viral infection, we have generated a recombinant non-replicative human adenovirus 5 (Ad5) expressing type I IFN-tau, a unique type I IFN with low toxicity and a broad host range in vivo. Using a mouse model of influenza infection, we have shown that a single-dose intranasal administration of recombinant Ad5-IFN-tau can effectively prevent lethality and disease induced by influenza virus. These findings show that our Ad5-IFN-tau might represent a safer option for first line antiviral treatments against zoonotic diseases.

Further, we have generated chimeric VLPs, derived from rabbit hemorrhagic disease virus (RHDV) for the presentation of foreign B-cell antigens, including a neutralizing epitope from feline calicivirus and an influenza A virus M2 protein epitope. The chimeric VLPs elicit potent protective humoral responses against displayed B-cell epitopes, as demonstrated by in vitro neutralization and in vivo protection against a lethal challenge. Additionally, a downsized versions of a synthetic dendrimeric peptide displaying two copies of a B-cell epitope linked to a T-cell epitope from foot-and-mouth-disease virus (FMDV) confers full protection against FMD virus challenge in pigs, pointing to a highly valuable, cost-effective FMDV candidate vaccine.

Lymphocystis disease affects marine and freshwater fish species worldwide and is caused by lymphocystis disease virus (LCDV). Using next-generation sequencing, we have identified within gilthead sea bream (S. aurata) lymphocystis lesions the concurrent presence of a new LCDV species (LCDV-Sa) as well as two novel viruses of the polyoma- and papillomavirus families. Because papillomaviruses have not been described in fish before, these findings support a more ancient origin of this virus family than previously thought.


The main objective of our group is to understand the regulation mechanisms of the immune response in fish, especially those triggered in response to viral infections. Our research pretends to use this increased knowledge on antimicrobial resistance mechanisms in fish to design more effective vaccines, immunomodulatory treatments and molecular adjuvants. Specifically, our research line is focused on the regulation of B lymphocytes, responsible for antibody production and dendritic cells implicated in the onset of adaptive immunity, as well as chemokines as mediators of leukocyte mobilization.

In the 2016-2017 period, we have studied how rainbow trout B cells are regulated in response to different stimuli. On one hand, we have compared the effects of IL6 to those of LPS, establishing that while IL6 is a differentiation factor for fish B cells, LPS leads the B cells to an intermediate stage in which they have increased antigen presenting capacities as well as an augmented antibody secretion. We have also identified an important role for CK9, a trout CC chemokine. CK9 has the ability of attracting B cells and macrophages and regulating innate functions in both cell types, such as for example phagocytosis. Additionally, we have studied the effects of BAFF, a cytokine produced in mammals by innate cells to regulate early B cell responses. In our studies, we have demonstrated that fish BAFF also affects different aspects of B cell biology, provoking increased survival and antigen presenting capacities. Interestingly, the effects observed on diverse subsets of B cells defined in both the spleen and the peritoneum were different for some of the functions studied. Surprisingly, on the contrary to the situation in mammals where only stimulated B cells produce BAFF, we also found that some specific subsets of B cells are capable of producing BAFF in homeostasis, in what seems as an auto-regulatory loop. Finally, we have identified the genes that code for the different BAFF receptors for the first time in teleost fish, and we have established a role for these receptors and their ligands in the pathogenesis of proliferative kidney disease (PKD) that goes along with an expansion of the B cell compartment, as well as in the expansion and differentiation of B cells that takes place in the peritoneum in response to an antigenic stimulation.
The general objective of the group is to advance in the knowledge of the Transmissible Spongiform Encephalopathies (TSEs) and the agent causing these diseases. Three lines of work are being developed: i) development of bioassay of high sensitivity for prion detection and diagnosis; ii) study of the elements involved in prion replication and pathogenesis and iii) study of the different prion strains in the different species and its capability of transmission to human.

During this period we have made further progress in the study of zoonotic potential of the diversity of prion strains in livestock species. We have also made progress in the knowledge of the elements that modulate prion transmission barrier between species. In this topic, the most important contribution has been the demonstration that persons with polymorphic variant PrP-Val129 are highly resistant to transmission of cattle BSE or BSE passaged in other species. However, PrP-Val129 individuals might be susceptible to infection with human-passaged BSE (vCJD) prions, and the propagated agents might transmit with molecular and neuropathological properties different from those of known vCJD in human (type 4 PrPres). These results indicate that human Val129-PrP polymorphic variant is a strong molecular protector against BSE zoonotic transmission but fails to prevent human-to-human vCJD transmission.

Our transgenic mouse models expressing different polymorphic variants of the PrPC in goat and sheep have allowed us to advance our understanding of the effect of some of these alleles in susceptibility/resistance to prion infection. We also showed that prions infectivity in water environments may remain for long periods (several years). These observations represent a great handicap for the control of these infectious diseases.

Other contributions allowed us to improve our understanding on some aspects of the pathogenesis of prion diseases and the potential role of the PrP in several physiologic and pathologic processes.
Our group aims to develop control strategies against arboviruses of interest in Animal Health as well as to establish animal infection models and virus detection tools. Our work has focused mainly on Rift Valley fever virus (RVFV) and bluetongue virus (BTV). Other viruses of interest are Crimean-Congo hemorrhagic virus (CCHFV) Schmallenberg virus (SBV) and African Horse sickness virus (AHSV). The group maintains a clear vocation for international collaborations in efforts to develop novel safer and efficacious control strategies for these viral diseases. Regarding BTV, the NS1 protein expressed by a modified vaccinia Ankara virus (MVA) vector can provide multiserotype protection against bluetongue virus that is largely dependent on CD8 T cell responses.

We have also engineered recombinant vaccine candidates based on proteins VP2, VP7, and NS1 of BTV-4 or VP2 and NS1 of AHSV-4 that were incorporated into avian reovirus muNS-Mi microspheres and rMVA. The combination of these two antigen delivery systems protected IFNAR (-/-) mice against lethal challenges with homologous and heterologous serotypes of BTV and AHSV. With respect to RVFV we have shown that treatment with silver nanoparticles greatly reduced the infectivity of RVFV. Regarding vaccine strategies, we tested the ability of homologous and heterologous DNA and MVA prime boost vaccination to induce and sustain immune responses in sheep. Furthermore, a bivalent MVA vaccine expressing BTV-NS1 and RVFV- GnGc induced protection against both virus infections in mice and reduced BTV and RVFV viremia in sheep. Also, a recombinant RVFV vector encoding BTV-VP2 or BTV-NS1 antigens have been rescued entirely from cloned cDNAs and we have identified point mutations reducing virulence in vivo but maintaining immunogenicity. Finally we have cloned and expressed CCHFV antigens in several expression systems (bacterial, insect and mammalian) for use in diagnostic and monoclonal antibody (mAb) production.

**SELECTED PUBLICATIONS**


