STAR-IDAZ
International Research
Consortium on Animal Health

Annual state-of-the-art report on animal health research on IRC priorities

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Contents

Executive summary **4**

I. RECENT INITIATIVES TAKEN TO SPEED UP R&D **5**

WHO R&D Blueprint 5

Coalition for Epidemic Preparedness Innovations (CEPI) 6

Innovative Medicines Initiative (IMI) 7

Zoonoses Anticipation and Preparedness Initiative (ZAPI) 8

Global Challenges Research Fund (GCRF) 9

Livestock Vaccine Innovation Fund (LVIF) 9

Innovative Veterinary Solutions for Antimicrobial Resistance (InnoVet-AMR) 9

Global Alliance for Livestock Veterinary Medicines (GALVmed) 10

Brucellosis vaccine prize 11

AgResults FMD Vaccine Challenge Project 11

II. INTERNATIONAL INITIATIVES TO FACILITATE TRANSNATIONAL R&D COLLABORATIONS **13**

Collaborative Working Group on European Animal Health and Welfare Research (CWG) 13

Global Strategic Alliances for the Coordination of Research on the Major Infectious Diseases of Animals and Zoonoses (STAR-IDAZ) 14

European Joint Programme (EJP) Co-fund on One Health (zoonoses – emerging threats) 15

Global research networks on specific diseases 16

International Veterinary Vaccinology Network (IVVN) 16

African Vaccinology Network (AfVANET) 17

Global coronavirus research and innovation network 17

III. RECENT INFRASTRUCTURES AND DATABASES TO FACILITATE R&D **19**

CWG Project Database 19

Disease Control Tools (DISCONTOOLS) 20

Veterinary Biocontained research facility Network (VetBioNet) 20

European Virus Archive goes global (EVAg) 21

Global Antimicrobial Resistance Research and Development Hub (Global AMR R&D Hub) 21

TRANSVAC 22

IV. STATE OF THE ART IN IRC PRIORITY DISEASES **24**

1. African Swine Fever (ASF) 25

2. Bovine tuberculosis (bTB) 29

3. Brucellosis 32

4. Foot-and-mouth disease (FMD) 34

5. Helminths 40

6. Porcine Reproductive and Respiratory Syndrome (PRRS) 44

7. Coronaviruses 47

8. Vector-borne diseases (VBD) 51

8. Antimicrobial resistance – Alternative to antibiotics 52

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More information on STAR-IDAZ IRC can be found at **www.star-idaz.net**

Disclaimer:

The report is a presentation of the current initiatives and recent scientific literature, organised to identify and highlight trends and advances in research on animal health, with a focus on priority animal diseases at a global level. The report does not target initiatives aimed at implementing animal disease control strategies (e.g., roadmaps for the control or eradication of infectious diseases) or at improving animal health control infrastructures.

Since the information relating to advances in animal health research is based on published articles, a time lapse between scientific breakthroughs and their publication is inevitable and so the report may not fully capture information on ongoing, or recently concluded, studies.

The findings and conclusions in this report are those of the contributors, who are responsible for the contents, and do not necessarily represent the views of the European Commission. Therefore, no statement in this report should be construed as an official position of the European Commission or of any of STAR-IDAZ IRC and SIRCAH members.

Executive summary

Introduction

The STAR-IDAZ International Research Consortium (STAR-IDAZ IRC) was established in 2016 to coordinate research activities at the international level, to speed up the development of new and improved animal health strategies for priority diseases/infections/issues of animals. The goal of the initiative is to deliver improved control tools and strategies, including candidate vaccines, diagnostics, therapeutics and other animal health products and procedures and/or key scientific information and tools to support risk analysis and disease control for at least 30 priority diseases by 2022.

The aim of this report is to provide STAR-IDAZ IRC Members, as well as other animal health stakeholders, with an overview of the existing opportunities for speeding up research and to boost collaboration in the sector, and to provide an overview of the latest discoveries on priority animal health diseases. Overall, this will support the decisions of policy makers and research funders, to accelerate coordinated development of control methods at the international level.

Methods

The first three Chapters of this report target recent initiatives taken to speed up R&D, to facilitate transnational R&D collaborations, and recent infrastructures and databases to facilitate R&D respectively. Information was collected by scanning the web with relevant keywords and collecting information from the SIRCAH partners.

The fourth Chapter reports on recent research developments on IRC priority diseases. For each disease, information about existing global research coordination networks is provided, and a collection of the main information on identified research gaps was derived from the DISCONTOOLS database. A selection of promising innovations or major research outcomes published in scientific journals between October 2019 and January 2021 were identified through a scan of the scientific literature in the CAB Abstracts database, using specific key words for each of the priority diseases/issues. This is completed with information on existing research efforts ongoing on the priority diseases/issues, collected from experts and research funders during STAR-IDAZ IRC Executive Committee, Scientific Committee, and Regional Networks meetings.

This report does not necessarily reflect the opinion of the STAR-IDAZ IRC members, but is the result of an analysis, by the Scientific Secretariat of the STAR-IDAZ IRC (SIRCAH), based on the collection of information from selected sources, including literature surveys.

I. RECENT INITIATIVES TAKEN TO SPEED UP R&D

Research and development (R&D) are fundamental to ensure the development of adequate disease prevention and control tools, as well as to make better use of knowledge that is currently available, and for modelling disease impact. Several initiatives have been started, at a regional or at a global level, to speed up research so as to deliver timely solutions to emerging issues.

The aim of this chapter is to provide a list of the main, recent funding and regulatory easing initiatives, and of the fast-track development pathways, which are designed to accelerate the delivery of R&D relevant to the animal health sector.

WHO R&D Blueprint

http://www.who.int/blueprint/en/

The World Health Organization (WHO) R&D Blueprint was established in 2016 as a global strategy and preparedness plan that allows the rapid activation of R&D activities during epidemics. Its aim is to accelerate the development and availability of effective tests, vaccines and medicines that can be used to save lives and avert large-scale crises.

A broad global coalition of experts from several medical, scientific, and regulatory backgrounds was convened by the WHO to contribute to the Blueprint. The World Organisation for Animal Health (OIE) serves as an observer in the Scientific Advisory Group of the initiative.

While the R&D Blueprint focuses on human diseases, most of the emerging human diseases are zoonoses, and thus the activity of this action could have positive impact on the control of animal diseases as well.

The activities of the R&D Blueprint will cover four main areas:

1. Improving coordination and fostering an enabling environment.
2. Accelerating R&D processes.
3. Developing new norms and standards tailored to the epidemic context.
4. Streamlined operational R&D response during outbreaks.

Among other activities, the R&D Blueprint will:

- Define and refine a robust and transparent semi-quantitative prioritisation methodology for infectious diseases most likely to create epidemics;
- Yearly update, using the prioritisation methodology described above, the list of diseases and pathogens to prioritise for research and development in public health emergency context;
- Develop a decision tree to assess the need for urgent R&D for potential emerging pathogens not yet included on the list; and
- Develop R&D Roadmaps and generic Target Product Profiles (TPPs) for priority diseases, through broad and open consultations with leading experts and other stakeholders.

One of the key components of the Blueprint is the delivery of R&D roadmaps to accelerate the development and implementation of effective medical countermeasures for WHO priority pathogens, aimed at reducing morbidity, mortality, and transmission. The current Blueprint priority pathogens are the following:

- Crimean-Congo haemorrhagic fever (CCHF);
- Ebola virus disease and Marburg virus disease;
- Lassa fever;
- Middle East respiratory syndrome coronavirus (MERS-CoV) and Severe Acute Respiratory Syndrome (SARS);
- Nipah and henipaviral diseases;
- Rift Valley fever (RVF);
- Zika;
- "Disease X".

Disease X represents the knowledge that a serious international epidemic could be caused by a pathogen currently unknown to cause human disease, and so the R&D Blueprint explicitly seeks to enable cross-cutting R&D preparedness that is also relevant for an unknown "Disease X" as far as possible. This definition fits well to describe COVID-19, which is now one of the priority pathogens for the Blueprint.

On February 11th - 12th 2020, the WHO R&D Blueprint held a workshop at the WHO Headquarters in Geneva, to enable identification of key knowledge gaps, and research priorities, on COVID-19, in order to accelerate the generation of critical scientific information and the most needed medical products to contribute to control the COVID-19 emergency. The high-level meeting involved more than 300 participants, including scientists (mostly from the human health side), country representatives, donors, and industry representatives from all regions.

The agenda was divided into 9 sessions, one of which was dedicated to "Animal and environmental research on the virus origin, and management measures at the human-animal interface", and co-organised with the World Organisation for Animal Health (OIE) and supported by the Food and Agriculture Organization of the United Nations (FAO).

As an outcome of the workshop, a research roadmap which defines priorities and governance framework addressing each of the thematic areas was published, and can be accessed at **https://www.who.int/blueprint/priority-diseases/key-action/Roadmap-version-FINAL-for-WEB.pdf?ua=1**.

A follow up virtual meeting was held on July 1st - 2nd 2020, to update the research roadmaps based on more recent scientific developments on the topic.

In September, the WHO issued a call for proposal for short projects (i.e., to be concluded by the end of February 2021) to fill in some of the identified research gaps at the human-animal-environment interface. Overall, fifteen projects have been funded, in different areas including investigation in animal populations, susceptibility of animals and virus evolution, virus detection, and behaviour of SARS-CoV-2 in the food environment.

Coalition for Epidemic Preparedness Innovations (CEPI)

http://cepi.net/

The Coalition for Epidemic Preparedness Innovations (CEPI) is an alliance, established in 2017, between governments, industry, academia, philanthropy, intergovernmental institutions, such as the WHO, and civil society. Its aim is to finance and coordinate the development of new vaccines to prevent and contain infectious disease epidemics, also ensuring that the vaccines to be developed will be affordable and available to populations with the most need. CEPI Secretariat is based in Oslo, Norway.

CEPI was founded by the governments of Norway and India, the Bill & Melinda Gates Foundation (BMGF), Wellcome, and the World Economic Forum. To date, CEPI has secured financial support from Australia, Austria, Belgium, BMGF, Canada, Denmark, the European Commission, Finland, Germany, Hungary, Italy, Japan, Kuwait, Lithuania, Luxembourg, Malaysia, Mexico, Netherlands, New Zealand, Norway, Panama, Saudi Arabia, Serbia, Singapore, Switzerland, United Kingdom, USAID, Ethiopia, The Republic of Korea, Indonesia, and Wellcome. Additionally, in 2020 CEPI received support from private sector entities as well as public contributions through the UN Foundation COVID-19 Solidarity Response Fund.

CEPI activities aim to:

- Stimulate, facilitate and finance the development of new vaccines against infections of epidemic potential, especially where pathways to regulatory approval and commercialisation are highly unpredictable;
- Advance candidate vaccines through the development process, so safety and efficacy are proved in principle through human trials, before epidemics begin. This will enable rapid full trials or emergency deployment in outbreaks;
- Establish the technical capabilities and processes necessary to accelerate research, development, manufacturing and clinical trials in the context of an outbreak;
- Work with industry, regulators and other bodies to ensure any vaccines developed get licensed and reach the people who need them; and
- Support the long-term development of epidemic vaccine preparedness within the countries most at risk from epidemic threats.

CEPI initially focussed on vaccines for known epidemic threats, selected based on the priority list of pathogens outlined in the WHO R&D Blueprint. In the first years of activity, the targets have been Middle East Respiratory Syndrome coronavirus (MERS-CoV), Lassa virus, Nipah virus, Rift Valley fever, Chikungunya and the so-called "Disease X" (i.e., a serious international epidemic caused by a pathogen currently unknown to cause human disease). Although CEPI's focus is on human diseases, most of the diseases in the WHO R&D Blueprint are zoonoses, and, in some specific cases, CEPI would consider the development of animal vaccines, as this would represent an effective way of controlling the control of the disease, preventing the development of human cases.

In 2020, CEPI activities has been mostly shifted toward COVID-19 response, and more specifically toward the development of human vaccines, initiating 9 partnerships to develop vaccines against COVID-19. The programmes will leverage rapid response platforms already supported by CEPI as well as new partnerships, with the aim of advancing COVID-19 vaccine candidates into clinical testing as quickly as possible.

Innovative Medicines Initiative (IMI)

http://www.imi.europa.eu/

The Innovative Medicines Initiative (IMI) is the Europe's largest public-private initiative aiming to speed up the development of better and safer medicines. IMI supports collaborative research projects and builds networks of industrial and academic experts to boost pharmaceutical innovation in Europe.

IMI was launched in 2008 and, to date, has an available budget of about €5.3 billion (€2 billion for 2008-2013 and €3.3 billion for 2014-2024). Almost half of this budget is provided 'in kind' by the EPFIA (pharmaceutical industry association) companies that are participating in the projects.

In 2020, a few weeks after the first reports of COVID-19 in the European Union, IMI launched a €45 million fast-track Call for proposals to develop therapeutics and diagnostics for current and future coronavirus outbreaks.

Currently, IMI has funded 168 projects (109 are in progress), with more still in the pipeline. While the main emphasis of these projects is on human health, some focus is on broad challenges in medical products development, such as drug and vaccine safety, the sustainability of chemical drug production, the use of stem cells for drug discovery, and antimicrobial resistance (AMR). One of these projects, Zoonoses Anticipation and Preparedness Initiative (ZAPI), financed in 2015, is specifically directed at zoonotic diseases, and this indicates that other initiatives in this area could be implemented in the future. More information about ZAPI could be found in the dedicated section in this Chapter.

Zoonoses Anticipation and Preparedness Initiative (ZAPI)

http://zapi-imi.eu/

The Zoonoses Anticipation and Preparedness Initiative (ZAPI) is one of the projects funded within the framework of the IMI public-private partnership. ZAPI aims to enable swift responses to major new infectious disease threats at the European and global levels, to be available within a few months after the first cases of the outbreak have occurred. It aims to do this by designing new manufacturing processes (up to large scale) for delivering effective control tools, such as vaccines, antibodies/antibody-like molecules, against (re-)emerging zoonotic diseases with pandemic potential.

ZAPI is a 5-year (2015-2020), €22 million, collaborative partnership between more than 20 European partners, including leading human and veterinary research institutions, non-governmental organisations, regulatory agencies, expert academic groups, and vaccine and biotech manufacturers. Boehringer Ingelheim Animal Health is the lead industry partner for vaccine development projects, and AstraZeneca is the lead industry partner for antibody products.

The ZAPI has three main objectives:

- To identify the best protective subunit vaccines and neutralising antibodies against potential new zoonotic diseases or strains of viruses, such as bunyaviruses or coronaviruses;
- To define optimum manufacturing technologies and processes for these vaccines and antibodies to enable high-volume production capacity; and
- To gain alignment with regulatory authorities and policy makers and secure pre-approval of the new vaccine and antibody manufacturing methodologies for future emerging zoonotic viral diseases.

ZAPI is focused on methods of rapidly delivering the products, rather than on their delivery itself. Its aim is full “development by design”, applicable to a wide range of pathogens that may emerge in future. The ‘Proof-of-principle’ for this approach will be obtained for the recently emerging target pathogens, RVF virus, Schmallenberg virus, and the MERS-CoV, which will be used as models. The approach is focussed on using molecular biology and protein modelling techniques to develop and characterise prototype vaccines and antibody products that employ virus-like particles to serve as carriers for various vaccine antigens.

The prototype ZAPI vaccine production platforms were primarily based on the use of recombinant baculovirus or yeast to produce large quantities of purified protein antigens in serum-free media, which are then ‘decorated’ onto the surface of a virus-like particle (‘multimeric protein scaffold particle’ - MPSP) using a proprietary protein-binding technology known as SpyTag/SpyCatcher. ZAPI research has demonstrated that this formulation could significantly enhance the immunogenicity of peptide antigens, and has other advantages, such as markedly improved thermotolerance, and ease of formulation for large scale production of vaccines.

To date, more than 40 scientific papers have been published from the work that has been conducted under this project. A ZAPI Final Stakeholders Global Meeting will be held virtually on February 4th – 5th 2021 to present an overview of the main findings and key learnings from the ZAPI project partnership’s experience for improving our One Health preparedness status for future pandemics.

Global Challenges Research Fund (GCRF)

https://www.ukri.org/research/global-challenges-research-fund/

The Global Challenges Research Fund (GCRF) is a 5-year (2016-2021) £1.5 billion fund, issued by the UK Government, aiming to support cutting-edge research that addresses the challenges faced by developing countries.

The GCRF developed a list of twelve priority challenge areas, falling under three main themes: 1. Equitable access to sustainable development, 2. Sustainable economies and societies, and 3. Human rights, good governance and social justice. Research on animal health and zoonoses can be included under several of the priority challenges, mainly those concerned with safe and resilient food systems supported by sustainable marine resources and agriculture, and sustainable health

The GCRF funding is awarded to UK researchers and to countries and territories eligible to receive official development assistance (ODA), which consist of all low- and middle-income countries based on gross national income per capita as published by the World Bank.

Several calls have been issued already, starting in 2016, and more than 500 projects have been funded at a global level.

Livestock Vaccine Innovation Fund (LVIF)

https://www.idrc.ca/en/initiative/livestock-vaccine-innovation-fund

The Livestock Vaccine Innovation Fund (LVIF) aims to bring together vaccine researchers, manufacturers and distributors, to accelerate the discovery of new vaccines and the improvement of existing solutions. The initiative concentrates on those animal diseases posing the greatest risk to subsistence livestock farmers/keepers in Sub-Saharan Africa, South and Southeast Asia, and targets transboundary diseases to achieve a lasting regional impact.

The LVIF is a five-and-a-half year (2015-2020), CA$57 million, partnership between BMGF, Global Affairs Canada and Canada's International Development Research Centre (IDRC). The initiative supports research into vaccine solutions, through a series of global competitive calls.

The fund has three main priorities:

- To accelerate the development of new vaccines against neglected livestock diseases by supporting innovation and leading-edge research,
- To increase the efficacy, marketability and use of existing livestock vaccines, and
- To foster effective partnerships between vaccine researchers and public and private sector actors to more efficiently develop, register, commercialise, and deploy livestock vaccines.

Innovative Veterinary Solutions for Antimicrobial Resistance (InnoVet-AMR)

https://www.idrc.ca/en/initiative/innovet-amr-innovative-veterinary-solutions-antimicrobial-resistance

The Innovative Veterinary Solutions for Antimicrobial Resistance (InnoVet-AMR) is a four-year, CA$27.9 million partnership between IDRC and the UK government's Global AMR Innovation Fund (GAMRIF) which is part of the Department of Health and Social Care (DHSC).

The aim of the initiative is to fund research that will develop innovative veterinary solutions focused on product development to reduce therapeutic and prevent non-therapeutic antimicrobial use in livestock and aquaculture production in low- and middle-income countries (LMICs). The programme specifically focuses on reducing AMR in swine, poultry, and aquaculture animals.

InnoVet-AMR, aims to achieve two main objectives:

- Support research that will identify innovative veterinary solutions, including vaccines and alternative solutions, to reduce the use of antimicrobials in livestock and aquaculture operations in LMICs;
- Build effective partnerships to better coordinate discovery, development and sustainable delivery of affordable innovative veterinary solutions to reduce the use of antimicrobials in livestock and aquaculture operations in LMICs.
- A call for proposals for "Developing innovative veterinary solutions for the fight against antimicrobial resistance" closed in 2018, and 11 projects were funded (four on poultry, four on fish and three on pigs; more information available at **https://www.idrc.ca/en/research-in-action/new-innovet-amr-projects**).

Global Alliance for Livestock Veterinary Medicines (GALVmed)

https://www.galvmed.org/

The Global Alliance for Livestock Veterinary Medicines (GALVmed) is a not-for-profit global alliance whose goal is to work with industry partners to reduce poverty and make a sustainable difference in access to veterinary medicines for small scale livestock farmers in sub-Saharan Africa and Asia. It is registered as a charitable foundation, with headquarters in Edinburgh and offices in Nairobi and New Delhi.

GALVmed focuses on the following three main areas.

- Research & Development: to support R&D for animal health products to meet specific needs of small-scale livestock producers in Asia and sub-Saharan Africa. Currently, the main projects involve animal African trypanosomiasis, brucellosis, foot and mouth disease (FMD), and smaller projects for cysticercosis, Newcastle disease (ND), and other diseases.
- Commercial Development: to establish sustainable markets to improve marketing and distribution channels for animal health products for use by small and medium scale producers in priority regions.
- Policy & Advocacy: current activities include advocating regulatory harmonisation in Africa, encouraging establishment of vaccine banks, supporting development of standards for veterinarians and veterinary paraprofessionals to improve access to veterinary services in low- and middle-income countries, and strengthening veterinary products regulatory systems, including improving regulatory controls to help eliminate substandard or falsified products.

GALVmed was formally established in 2005, with initial funding from the UK Government Department for International Development (DFID). By 2008, funding from BMGF and the UK Government enabled GALVmed to commence programmes of delivery. Since 2008, GALVmed has received over $100 million in donor funding for programmes in pursuit of its mission.

To date, GALVmed funded programmes have targeted the development of new products (veterinary vaccines, pharmaceuticals, and diagnostics) and various product improvements (such as heat tolerance, production cost reductions, formulations for easy applications), as well as the development of sustainable access to these products.

Current GALVmed projects include a 'Tryps 2' project to develop and commercialise a pharmaceutical treatment for trypanosomiasis, and two AgResults competitions that GALVmed is managing where the goal is to incentivise development, registration, and commercialisation of new or improved vaccines against brucellosis and FMD by offering prizes for successful completion of various milestones rather than offering up-front research grants.

Additional information about the two AgResults competitions can be found in the two dedicated sections in this Chapter.

Brucellosis vaccine prize

https://brucellosisvaccine.org/

The Brucellosis Vaccine Prize is a US $30 million prize competition that invites vaccine developers ('solvers') to submit their proposals for developing a suitable vaccine that is efficacious, safe and viable for use against *Brucella melitensis* in small ruminants across the developing world. This global competition is funded by AgResults (a collaborative initiative between the governments of Australia, Canada, the UK and the USA, as well as the Bill & Melinda Gates Foundation), and implemented by the Global Alliance for Livestock Veterinary Medicines (GALVmed).

The competition is open to any animal health, biotechnology, or pharmaceutical company, and other organisations. It is structured in three phases:

- Phase 1 ('Application Phase'): solvers are invited to submit their initial application to participate in the Competition (deadline November 18, 2017). The first Milestone Payment is a one-off payment of US $100,000, which may be awarded to a maximum of ten participants.
- Phase 2 ('Solving Phase'): solvers can work towards the production of a proof-of-concept together with other deliverables (which are outlined in the official Competition Rules document). This phase can start for each Solver upon successful application and leading up to the potential award of Milestone Payment 2 (US $1,000,000; up to a max of 4 solvers).
- Phase 3 ('Final Phase'): solvers will be required to take their vaccine candidates from their 2nd Milestone Deliverables to a registered product. This phase can start for each solver upon successful application and completion of Phase 2 and leading up to the potential award of the Grand Prize (US $20,000,000) or Best in Class Prize (US $5,000,000). The competition will close on November 2026.

Phase 1 is now concluded, and 10 participants have already been awarded the first Milestone Payment; the competition is now in Phase 2 (15 participants have entered this phase). For organisations that have been accepted into Phase 2 of the competition, the primary focus will be to demonstrate proof of principles of efficacy and safety, and establish a scaled production process, together with other deliverables. The competition will probably be extended until 2022 to complete Phase 3.

'Pull mechanisms' such as this prize, rewarding research output rather than research input, represent an innovative way to stimulate applied, product-oriented, research into neglected diseases.

AgResults FMD Vaccine Challenge Project

https://www.galvmed.org/foot-and-mouth-project/

In January 2020, AgResults kicked off a new competition for encouraging the development and uptake of an improved vaccine specifically for the needs of East Africa, called the Foot and Mouth Disease (FMD) Challenge Project. The project is supported by Australia, United Kingdom, Canada, USA, BMGF, and the World Bank, and is led by GALVmed.

The FMD Vaccine Challenge Project will encourage pharmaceutical companies around the world to develop, register, and commercialise effective vaccines for the control of FMD in East Africa. These companies will participate as "competitors" to create vaccines that meet criteria established for the region. Once the vaccines are approved and registered, competitors will become eligible to commence sales.

The project will contribute to the cost-per-dose paid to the competing manufacturers, thereby encouraging government and private sector actors to better combat FMD by consistently purchasing high volumes of vaccines at affordable prices. To build a stable market around FMD control, the project will promote the development of a private sector model for buying and distributing vaccines, while enhancing existing public sector control efforts. As the market develops, the project plans to expand access to effective vaccines among smallholder farmers, yielding improvements in livestock health and increases in net income.

The competition has two phases, a Development Phase followed by a Cost-Share Phase. During the Development Phase, Competitors must submit FMD vaccine dossiers (that they believe meets all of the elements of the AgResults Target Product Profile) to the regulatory authorities in at least two target countries, by August 2023. If no dossiers are submitted by this date, AgResults reserves the right to terminate the competition. If a dossier is submitted for assessment by the deadline, that Competitor will be eligible to submit an application to AgResults (via the online portal) upon receipt of full product registration in at least two target countries. The Cost-Share Phase will start once the first vaccine is approved by AgResults and the Competitor has received order(s) of at least 150,000 doses.

Once the first vaccine has been approved by AgResults, other Competitors will still be eligible to submit applications (until 3.5 years after the start of the Cost-Share Phase).

The Request for Applications document, containing full details of the Competition, was published in July 2020 and can be accessed at **https://www.galvmed.org/wp-content/uploads/2020/09/FMD-Vaccine-Challenge-Project-RFA-FINAL-rev1-010920.pdf**.

GALVmed is conducting a first round of data collection and plans to share a preliminary report in early 2021.

II. INTERNATIONAL INITIATIVES TO FACILITATE TRANSNATIONAL R&D COLLABORATIONS

The progressive reduction of public funding, as well as the increasing threat of emerging diseases, creates the pressing need to prioritise research topics and to prevent unnecessary duplication of research. Increasing transnational collaboration in research would help addressing these needs.

The aim of this chapter is to provide a list of the main recent and/or ongoing initiatives designed to improve and facilitate the international and transnational collaboration in animal health research.

Collaborative Working Group on European Animal Health and Welfare Research (CWG)

http://www.scar-cwg-ahw.org/

In 2005, in response to an initiative of the EU Standing Committee on Agricultural Research, the Collaborative Working Group on European Animal Health and Welfare Research (CWG) was established. The aims of this group, encompassing representatives of funding bodies from over 20 European countries, were the sharing of information, coordination of research activities, and the definition of a common research agenda.

Several actions have been initiated in the EU under the auspices of the CWG, with the aim of improving transnational collaboration in research and to start a European coordination of research to define a coherent European research area. Building on this framework, networks between research funders on animal health were supported through four EU funded initiatives, the EMIDA ERA-NET (European Research Area Network on Emerging and Major Infectious Diseases of Livestock 2008 - 2011), the STAR IDAZ Global Net (Global Strategic Alliances for the Coordination of Research on the Major Infectious Diseases of Animals and Zoonoses, 2011 - 2015), the ANIHWA ERA-NET (European Research Area Network on Animal Health and Welfare, 2011-2015), and the ICRAD ERA-NET (International Coordination of Research on Infectious Animal Diseases, 2019 - 2023). Today, the CWG also acts as European Regional Network for the STAR-IDAZ IRC.

The CWG has continued to hold biannual meetings since it was formed.

In April 2020, in response to the COVID-19 pandemic, the CWG decided to implement (jointly with the STAR-IDAZ IRC) a survey among its members to investigate about their current and planned research on coronaviruses (CoVs), and on their interest in a common research call on this subject.

The survey specifically targeted research on the development of vaccines, diagnostics, and therapeutics, as well as studies on host-pathogen interactions, epidemiology, and control of a number of CoVs of interest for the animal health sector, or with a zoonotic potential. These were:

- Porcine epidemic diarrhoea virus (PEDV), transmissible gastroenteritis virus (TGEV) and other coronaviruses of pigs;
- Avian infectious bronchitis virus (IBV) and other avian coronaviruses;
- Middle East Respiratory Syndrome CoV (MERS-CoV);
- Severe acute respiratory syndrome CoV 2 (SARS-CoV-2);
- Other coronaviruses of domestic animals (including companion animals);
- Bat coronaviruses;
- Coronaviruses in general, including model systems.

The exercise identified a few areas of common interest for several funders and was used as a basis for discussion about the possibility of launching a common CWG call on CoVs. Several rounds of discussion were held with interested funders, who decided that instead of launching a CWG call, some of the topics of interest identified by the survey should be funded as part of a next call of the ICRAD ERA-NET (described separately in Section II of the Report). The call is planned to be launched in 2021.

In addition, on the 30th June 2020, the CWG and the STAR-IDAZ IRC jointly organised a global interactive webinar titled "PANDEMIC! A one health view of emerging infectious diseases. What veterinary sciences can contribute". The webinar presented the lessons learnt from the COVID-19 pandemic and aimed to investigate the role of veterinary sciences in coping with, and preventing, future animal and zoonotic pandemics. It discussed how research on animal infectious diseases can contribute to preventing and controlling future human and animal pandemics and how Strategic Research Agendas in animal health can be improved to respond to emerging animal diseases. The event was structured in two phases, with invited talks from Ilaria Capua (University of Florida, USA), Linfa Wang (Duke-NUS Medic School, Singapore), Jean Christophe Audonnet (Boehringer Ingelheim, France) and Mark Woolhouse (University of Edinburgh, UK) and an overview of the STAR-IDAZ IRC by Alex Morrow (Defra, UK) to summarise the current knowledge. This was followed by a round table discussion with experts, including Arjan Stegeman, Utrecht University; Jean Charles Cavitte, European Commission, DG AGRI; Hein Imberechts, Coordinator One Health EJP; and Luke O'Neill, Trinity College Dublin, Ireland. Webinar attendees were invited to participate in the round table discussion by responding via an interactive web tool to specific questions. The event was attended by 506 participants from academic and public research institutions, industry, veterinary services, and other government bodies from 53 countries. A meeting report, summarising the main meeting outcomes and considering inputs from the experts during the invited talks and the round table discussion, as well as from the audience, was published after the webinar. It outlines identified research needs and policy recommendations for the prevention and control of human and animal pandemics. These can give a new direction to Strategic Research Agendas on animal health and promote collaboration between the different actors.

The workshop was recorded and can be viewed **here**.

The full report of the meeting can be accessed **here**.

Global Strategic Alliances for the Coordination of Research on the Major Infectious Diseases of Animals and Zoonoses (STAR-IDAZ)

http://www.star-idaz.net/

The "Global Strategic Alliances for the Coordination of Research on the Major Infectious Diseases of Animals and Zoonoses" (STAR-IDAZ) was a four-year (2011-2015) FP7 project aiming to extend the coordination of animal disease research at a global level.

The aims of STAR-IDAZ were to strengthen the linkages between and reduce the duplication of global research effort, maximise the efficient use of expertise and resources and accelerate coordinated

development of control methods at the international level. To achieve this, an international forum of R&D programme owners/managers and international organisations was established to share information, improve collaboration on research activities and work towards common research agendas and coordinated research funding on the major animal diseases affecting livestock production and/or human health.

The scope of the project included coordination of research relevant to emerging and major infectious diseases of livestock, including fish and managed bees, and those infections of livestock that carry the risk of disease threat to human health. Diseases of wildlife were also considered where they were identified as reservoirs of infection with emerging and major infectious diseases of humans or production animals.

The aims of STAR-IDAZ were to:

- Strengthen the linkages between and reduce the duplication of global research effort on high priority infectious diseases of animals (including zoonoses) maximise the efficient use of expertise and resources and accelerate coordinated development of control methods;
- Identify and co-ordinate the response to gaps in research activities for targeted diseases;
- Create the necessary critical mass and capacity to address emerging infectious disease threats;
- Improve the cost–effectiveness and added value to network partners of current research programmes;
- Develop durable procedures for a better co-ordinated, rapid response to urgent research needs;
- Identify unique regions with localised diseases and improve access to research in those areas; and
- Improve access to and the utility of research results across all partner organisations.

STAR-IDAZ was successful in establishing, through its global and regional activities, a network of organisations managing research budgets or programmes in approximately 50 countries that are committed to working together. Since 2015, the network moved forward as a self-sustaining network under an agreed Memorandum of Understanding with most partners signing up to a higher level of commitment in STAR-IDAZ International Research Consortium. Since 2016 it is supported by a Secretariat (SIRCAH) funded by the European Commission.

European Joint Programme (EJP) Co-fund on One Health (zoonoses – emerging threats)

https://onehealthejp.eu/

The European Joint Programme (EJP) Co-fund on One Health (zoonoses – emerging threats) is a 5-year (2018-2023), €90 million, initiative aiming to create a European joint programme to deal with "one health" issues, primarily targeting food-borne zoonoses and antimicrobial resistance, and, to a lesser extent, emerging zoonotic threats. The project Consortium includes 39 public research institutes from 19 European countries. In order to ensure a One Health approach, a balanced number of human/public health and veterinary institutions are included. An enlargement campaign is currently underway, to increase the number of Member States involved in the project (today, 17/27, plus UK and Norway), and to have both the human health and animal health sectors represented in the consortium for every member country.

The EJP aims to build a sustainable framework for an integrated community of research groups including reference laboratories in the fields of life sciences, medicine, veterinary medicine, animal sciences, food sciences and environmental sciences. Integration and alignment in research will be improved through funding of research projects. Two research calls have been launched, and a total of 24 projects have been funded. A new project was proposed in September 2020 to target the development and harmonisation of detection and characterisation methods for SARS-CoV-2 in humans and animals (lead by Wageningen Bioveterinary Research and the University of Surrey, with another 19 partners). The discussions with the EU Research Executive Agency (REA) and its Steering Group on the implementation of this proposal are still continuing.

In addition to traditional research projects, the EJP funds integrative projects to develop common protocols or infrastructure that support collaborative processes (e.g., platforms for uploading, sharing and analysing sequence data, experimental facilities or risk assessment structures), as well as PhD students, Summer Schools, and Short-Term Missions, and implemented numerous integrative activities.

Global research networks on specific diseases

(websites link for the specific networks, when available, are provided in Chapter IV)

The sharing of information and scientific knowledge is of paramount importance to ensure disease preparedness. To this end, global research networks and alliances have been established on a number of infectious diseases to exchange and generate knowledge that would support the development of tools to successfully prevent, control or eradicate such diseases.

Although these networks present slightly different objectives, the identification of research needs and the coordination of research on priority issues are common activities.

To date, such networks exist for a number of diseases such as ASF, animal influenza, bovine tuberculosis, helminths, and FMD. A project call for establishing one for coronaviruses has been preannounced for early 2021, and is described separately in this Section.

Further details on the specific networks for the other STAR-IDAZ IRC priority diseases are provided in Chapter IV.

International Veterinary Vaccinology Network (IVVN)

http://intvetvaccnet.co.uk/

The International Veterinary Vaccinology Network (IVVN) is a multidisciplinary and inter-connected vaccinology research and development community. It aims to address the challenges impeding vaccine discovery, as well as evaluation and delivery of vaccines that will have impact on the control of priority livestock and zoonotic diseases in low-and-middle income countries (LMICs). Built on the basis of the UK Veterinary Vaccinology Network, the IVVN has to date more than 1,000 members.

The objectives of the IVVN are to:

- Establish an interactive and multi-disciplinary Network to facilitate dissemination of knowledge and exchange of 'state-of-the-art' technology between members of the veterinary (and human) vaccinology communities;
- Identify and fund collaborative teams with complementary expertise that through application of novel approaches can effectively address critical 'bottle-necks' in vaccine development for LMICs-relevant pathogens;
- Advance the development of veterinary vaccines for LMICs-relevant diseases;
- Provide the scientific and logistical support for members to secure substantive funding to expand on the preliminary data generated by pump-priming funding; and
- Engage with a variety of industry partners, in both developed and LMICs, to ensure the sustainable delivery of effective vaccines.

The IVVN facilitates collaborations between scientists, industrial partners and others from the UK and LMICs across the broad range of disciplines that can contribute to vaccine development, by funding scientific meetings, workshops, laboratory exchanges and supporting 'pump-priming' projects. Awards of up to £100,000 are available to support pump-priming projects from collaborative teams of IVVN members, which address a key bottleneck preventing the development of a vaccine for livestock and zoonotic diseases of importance in LMICs. The IVVN have awarded funding to 13 projects over four rounds of funding, the last of which was announced in April 2020. Laboratory exchange funding Awards of up to

£10,000 are available to support transfer of expertise between laboratories within the Network, or to fund a proof-of-concept piece of work. The IVVN have funded 13 projects over three rounds of funding.

Due to the COVID-19 pandemic, the IVVN third annual conference, initially planned for Vietnam, has been postponed to 2021, while several other planned workshops were suspended, forcing IVVN to begin considering how it can best continue to support the global network that forms its community. As part of its future strategy, IVVN will start an online webinar series to give its members across the globe the opportunity to hear from experts in a variety of areas of vaccine research. In addition, IVVN is working to establish a new database that will integrate a wealth of diverse data to support veterinary vaccinology researchers. This database will contain information about specialised vaccine research resources and knowledge, highlight groups with pathogen-specific expertise, hold information about current research activities and also information about industrial and academic partners.

African Vaccinology Network (AfVANET)

http://afvanet.org/

The initiative to establish the African Vaccinology Network (AfVANET) was taken in 2016, when a group of African researchers met, on the side of a symposium on 'New approaches to vaccines for human and veterinary tropical diseases', to discuss the need for a better involvement of African scientists in finding solutions to infectious diseases that negatively impact the health and the economy of the continent.

Through this initiative, African researchers will be able to take the necessary initiatives to solve the problems of their continent and provide appropriate solutions that are in most cases different from region to region.

The goals of this platform are to:

- Bring together all stakeholders in vaccinology and related sciences in Africa;
- Identify and prioritise vaccine gaps in Africa;
- Promote vaccine research and development in Africa; and
- Promote sound ethics, biosafety and biosecurity in Africa.

The kick-off meeting of the AfVANET took place on 19-20 March 2019 in Nairobi, Kenya. Around 30 speakers, both from the human and animal health sectors, attended the meeting, coming from Africa, Asia, Australia, and Europe.

Global coronavirus research and innovation network

https://www.ukri.org/opportunity/global-coronavirus-research-and-innovation-network/

The Biotechnology and Biological Sciences Research Council (BBSRC) and Defra are making £500,000 available to fund a Global Coronavirus Research and Innovation Network. This will be a coordinated global One Health network of researchers and stakeholders producing evidence and innovation that enables the better understanding and development of intervention tools and strategies for animal and human coronaviruses. The network aims to establish and sustain global research and innovation partnerships to generate knowledge, tools, and intervention strategies for control of animal and human coronaviruses. The proposals should seek to coordinate research in pathogen biology (including the seasonality, transmission, ecology, and evolution of coronavirus), host response and effective intervention strategies. The pre-announcement came out on 25th November 2020 and the call was published on the 4th January 2021. The decision on funding will be made in March 2021 and the network is expected to start in early May.

Following the COVID-19 pandemic, significant funding has gone into CoVs: this network will aim to identify the research gaps and where research should focus in order to inform future funding of the area. The network will i) facilitate research collaborations and serve as a communication gateway for

global human and animal coronaviruses research community, ii) conduct strategic research to better understand coronaviruses, iii) aim to develop the next generation of control measures and strategies for their application, iv) determine social and economic impacts of new generation improved intervention strategies, v) provide evidence to inform development of socio-economic policies, and vi) integrate human, animal, and environmental health – in a One Health approach. The funding is available for hiring a network manager, bringing the research community together supporting meetings or conferences, and set up a website.

The network will also be encouraged to align with existing networks, for example the Global Virus Network and the STAR-IDAZ IRC. The aim will be to keep it very open and inclusive to encourage new members throughout the life of this network. It will be a similar concept to existing global research alliances in the animal health area (such as the Global ASF Research Alliance – GARA, and the Global FMD Research Alliance – GFRA), with the main difference being they are self-funded, and this network has funding provided by Defra and BBSRC.

III. RECENT INFRASTRUCTURES AND DATABASES TO FACILITATE R&D

Conducting scientific research requires significant research infrastructure, including facilities, resources and related services. The establishment of common databases, allowing the sharing of knowledge and facilitating networking, is of paramount importance to facilitate and accelerate R&D.

The aim of this chapter is to provide a list of the main distributed infrastructure and databases relevant to the animal health sector.

CWG Project Database

http://database.scar-cwg-ahw.org/

The Collaborative Working Group on European Animal Health and Welfare Research (CWG) was established in 2005 to increase information sharing and research coordination in the European area. To meet these objectives, as one of the objectives of the EMIDA project, a framework was established under the CWG to capture research project information. From this a database was developed, to collect information on funded projects on animal health. This database was further updated under the ANIHWA project, to also collect projects on animal welfare supported by CWG funding bodies. This was expanded under STAR-IDAZ to include project data from organisations outside of Europe.

To date, details of over 2,340 projects (both national and international) have been uploaded to the project database by the project partners. The projects can be searched according to research area, disease, pathogen, animal species, country, end date and by full text.

This database represents a valuable tool to map current research on animal health, to allow research funders to identify areas where investments in research are lacking and to avoid duplications.

As mentioned in the section dedicated to CWG in Chapter I of this document, in 2020 the CWG conducted a survey to collect information about research projects being currently funded or planned on CoVs by the members' organisations and funding bodies. The collected information was shared back with the STAR-IDAZ IRC and CWG members and is now stored in the STAR-IDAZ IRC website members' area, complementing the information collected by the CWG project database on this area.

Disease Control Tools (DISCONTOOLS)

http://www.discontools.eu/

DISCONTOOLS (DISease CONtrol TOOLS) is an open-access database to assist public and private funders of animal health research and researchers in identifying research gaps and planning future research. The database contains research gaps as well as a gap scoring and prioritisation model for more than 50 infectious diseases in animals. The data are provided by disease-specific expert groups, reviewed by a project management board, and updated in a 5-year cycle. Users can select their topics of interest, compare the selected topics across diseases and prioritise the diseases according to a range of customisable criteria. By identifying the gaps in knowledge and available control tools, DISCONTOOLS helps to prioritise research and speed up the development of new diagnostics, vaccines, and pharmaceuticals.

DISCONTOOLS is funded by a consortium of members from the European Collaborative Working Group on Animal Health and Welfare Research (CWG), with industry providing secretariat support. The website received a facelift in 2018 and has become an important resource for funders of animal health research and the research community to develop research agendas and evaluate research proposals. Recently, DISCONTOOLS published an **e-book** with one-pagers on the gaps in control tools and research needs for 53 animal diseases. Depending on when COVID-related measures are relaxed, a symposium will be organised in Brussels with all relevant stakeholders, to review key research priorities in animal health and start a new cycle of updating the listed diseases.

Veterinary Biocontained research facility Network (VetBioNet)

http://www.vetbionet.eu/

VetBioNet (Veterinary Biocontained research facility Network) is a project funded under the European Commission Horizon 2020 Research Framework for large research infrastructures (2017-2021). The project consortium includes 30 academic and industrial partners from 14 countries across Europe, Africa, and Oceania. VetBioNet's principal objective is to strengthen European capacity and competence to meet the challenges of emerging infectious diseases by reinforcing the network of European BSL3 (Biosafety Level 3) infrastructures dedicated to livestock. It will serve as a multidisciplinary network seeking to drive the European R&D agenda related to emerging epizootic and zoonotic diseases. Moreover, it will develop new technologies as well as activities such as standardisation of protocols and best practices and facilitate connecting with similar institutes outside Europe.

To reach its overall objectives, VetBioNet will:

- Promote and facilitate Transnational Access (TNA) to the infrastructure resources of the network, including BSL3 animal experimental facilities and laboratories, technological platforms, and sample collections;
- Promote technological development by involving private partners in the integrating activities of the network and by providing a communication platform for bidirectional exchange with industry stakeholders (Stakeholder Platform);
- Enhance the preparedness of the major European BSL3 research infrastructures to accelerate the response to (re)emerging epizootic and zoonotic threats by sharing capacities beyond the infrastructures;
- Harmonise Best Practices and promote the use of global standards in European BSL3 infrastructures;
- Forge cooperative relationships with non-European BSL3 infrastructures, research institutes, industrial partners, international organisations, and policy makers;
- Ensure high ethical standards and clarify the social impact of VetBioNet research work;
- Develop and implement a Sustainability Plan for the network to continue beyond the five-year term of funding; and
- Carry out Joint Research Activities (JRAs) designed to improve the scientific and technological standards of the integrated services provided by the network infrastructures.

The project is structured around three types of activities: transnational access (TNA), networking activities and joint research activities (JRAs). TNA provides free-of-charge access to the BSL3 facilities, technical resources, and sample collections of VETBIONET consortium members, via an ongoing research call process and through a dedicated web portal. The JRAs include programmes optimising the modelling of epizootic and zoonotic diseases in animals, based on in vitro cell, tissue and organ cultures.

Among the results achieved so far on the development and optimisation of livestock infection models, three disease models have been finalised. These involve the standardisation of infection trials with peste-des-petits-ruminants (PPR) virus in sheep, poultry infection trials with both avian influenza virus and with Salmonella, and the development of trout and carp disease models for the study of viral fish diseases.

To support its integrated activities, VETBIONET has developed accredited training courses with the Federation of European Laboratory Animal Science Associations and other organisations. The first training school took place in January 2019.

The project is harmonising protocols and best practices, establishing guidelines to help upgrade high-containment facilities and promoting the use of global standards in all European BSL3 facilities, with a particular focus on animal welfare and alternatives to animal experimentation.

European Virus Archive goes global (EVAg)

https://www.european-virus-archive.com/

The European Virus Archive (EVA) project was funded under the European Commission FP7 (2009-2014) to create and mobilise a European network of high calibre centres with the appropriate expertise, to collect, amplify, characterise, standardise, authenticate, distribute, and track, mammalian and other exotic viruses. The network produced associated reagents on demand, to laboratories, mainly throughout Europe. In 2015, a new project was awarded under the Horizon 2020 Programme to enlarge the archive and make it global (EVAg, 2015-2019).

Today, EVAg is a non-profit organisation dedicated to the characterisation, conservation, production, and distribution of biological materials in the field of virology. Its global virus collection is a valuable support tool for the organisation of scientific research, education, and disease control through human and veterinary health programmes, providing both essential resources as well as a platform for the continuation of project-derived products.

The EVAg consortium includes an international group of 26 laboratories, 17 belonging to EU Member States' institutions and 9 to non-EU institutions, and a number of Associated Partners (to date, 14 institutions from 11 non-EU Member States and 3 EU Member States), all sharing the common interest of creating an international virus collection.

In 2020, EVAg joined the struggle against COVID-19, building a new section in its portal dedicated to information concerning preclinical evaluation of molecules with antiviral potential against the SARS-CoV-2, and making available on its catalogue SARS-CoV-2 biological material (full virus, viral proteins, nucleic acids and diagnostics tools).

Global Antimicrobial Resistance Research and Development Hub (Global AMR R&D Hub)

https://globalamrhub.org/

The Global Antimicrobial Resistance Research and Development Hub (Global AMR R&D Hub) was established, in May 2018, in response to the Joint Statement of Intent of the G20 Focal Points of the G20 Health Working Group. It called for the setting-up of a new, international R&D collaboration hub in the field of antimicrobial research and product development aimed at maximising the impact of new and existing initiatives in basic and clinical antimicrobial research, as well as product development.

The main goal of the Global AMR R&D Hub is to promote high-level coordination among governments and upstream funders from different world regions, to better align national and international efforts in the fight against AMR. Its scope is embedded in a comprehensive One Health approach relating to R&D on AMR, comprising human and animal health as well as environmental aspects.

The central deliverable of the Global AMR R&D Hub is a close to real-time Dynamic Dashboard (**https://dashboard.globalamrhub.org/**) providing information and analysis at a high level on current initiatives, funding flows and activities in the field of AMR R&D. The dashboard presents pre-analysed information, to inform policy makers in their decision making on strategic investments and actions in AMR R&D. While the Dashboard initially covered only bacterial infections for humans, in 2020 it was enlarged to include information on AMR R&D related to animal health too. The OIE and the STAR-IDAZ IRC, along with other international experts, have supported the Hub in identifying animal health-specific categorisation fields for the implementing this new feature of the Dynamic Dashboard. The animal health part of the Hub already contains information on 1084 projects from 89 funders worldwide, for a total budget of over US $660 million.

In 2020, the Global AMR R&D Hub entered a formal collaboration partnership with the STAR-IDAZ IRC, with the aim of cooperating on their common interest to strengthen global research efforts and reduce duplication of research on priority infectious diseases of animals relevant to AMR. This joint effort includes the exchange and dissemination of relevant AMR R&D expertise, information and data in animal health and supports more efficient use of international resources through the identification of gaps, overlaps and potential synergies.

In December 2020, the Hub held a Virtual Conference named “Translating AMR R&D mapping into policy and action”, which aimed to start translating the AMR R&D information presented in the Global AMR R&D Hub’s Dynamic Dashboard into policy and action. Key outcomes, ideas and discussion points raised at the conference will be referred to the Global AMR R&D Hub’s Board of Members (which is composed of policy makers, funders and international organisations) to be considered in their recommendations on where more action is needed. The event was built around three sections, one of which was entirely dedicated to the animal health field (Session 2, “Filling AMR R&D gaps in animal health at country, regional and global level”). A full meeting report is available at **https://globalamrhub.org/wp-content/uploads/2021/01/Conference-report-and-recommendations_v2.pdf**

TRANSVAC

https://www.transvac.org/

The European Commission, in the context of the Horizon 2020 Framework Programme, has recently funded “TRANSVAC2”, a European vaccine R&D infrastructure (2017-2022; 10.6 million euros). The aim is to facilitate access to skills and capacities, and to promote collaborations in the European vaccine landscape, to accelerate the development of safe, effective, and affordable vaccines.

TRANSVAC2 builds upon the success of TRANSVAC (the European Network of Vaccine Research and Development) funded under the EC´s previous Framework Programme (FP7). The first TRANSVAC project made significant contributions to the European vaccine development landscape, providing scientific-technical services to more than 29 vaccine projects and developing a roadmap for the establishment of a sustainable European vaccine R&D.

The main goal of TRANSVAC2 is to support innovation for vaccine development. High quality technical services across four different service platforms are offered: i) Technology (for process development and Good Manufacturing Practices (GMP) production), ii) Immunocorrelates and Systems Biology, iii) Animal models, and iv) support for Clinical Trials.

Academic and non-academic research groups, including SMEs, can apply to benefit from the expertise, reagents, and facilities offered by TRANSVAC2 to accelerate the development of their vaccines.

TRANSVA2C also offers training courses to provide fundamental and advanced knowledge on a wide-range of vaccine development-related topics. Training modules will be harmonised with existing European vaccinology courses, aiming to complement existing infrastructures and activities. TRANSVAC2 will therefore centralise and expand the training opportunities available to the European vaccine community.

With this comprehensive approach, TRANSVAC2 functions as a leverage and innovation catalyst between all stakeholders involved in vaccine R&D in Europe, and contributes to the development of effective products to address European and global health challenges. This reinforces the European leadership in controlling the burden and spread of diseases, and the economic assets represented by vaccine developers in Europe.

TRANSVAC2 seeks to support vaccine-related projects currently in the preclinical phase of development. It launched two calls in 2020 (June and October) and plans to launch two additional calls in 2021 (February and June). TRANSVAC2's joint research activities (JRAs) aim to address current major gaps in vaccine development knowledge and are designed to feed directly into and to support the transnational activities. The main focus of such activities is improving adjuvants, predictive assays, systems biology, and animal models.

IV. STATE OF THE ART IN IRC PRIORITY DISEASES

In the framework of the STAR-IDAZ project, a list of priority diseases and crosscutting issues for which research coordination is required to make progress and deliver the control tools that are needed was identified. This preliminary list was further discussed during the meetings of the STAR-IDAZ IRC Executive and Scientific Committees' meetings held between 2017 and 2020 and updated accordingly. The full list of the currently identified priorities is reported below.

- African Swine Fever (ASF)
- Animal genomics/genetics for animal health
- Antimicrobial resistance (AMR) and the Development of Innovative Alternatives to Antibiotics
- Bovine Tuberculosis (bTB)
- Brucellosis
- Coronaviruses (CoVs)
- Diagnostics (tools and technologies)
- Emerging issues
- Epidemiology
- Foot and Mouth Disease (FMD)
- Foresight
- Helminths
- Vaccinology
- Influenza
- Mastitis
- Mycoplasmas (including contagious bovine pleuropneumonia – CBPP and contagious caprine pleuropneumonia - CCPP)
- One Health
- Porcine Reproductive and Respiratory Syndrome (PRRS)
- Porcine Respiratory Disease Complex (PRDC)
- Pox virus infections
- Vector-borne diseases (VBD)

In the framework of the STAR-IDAZ IRC Executive Committee meeting that was held in Kenya (2017), the first six diseases/issues to be addressed were selected. These were: ASF, bTB, brucellosis, FMD, helminths, and PRRS. During the STAR-IDAZ IRC Executive Committee meeting, held the following year (2018) in Spain, CoVs and VBD were selected as additional topics to be addressed. At the STAR-IDAZ IRC Executive Committee meeting held in China in 2019, the scope of the work to be performed on VBD was better defined, and it was decided to start working on antimicrobial resistance (AMR) and the development of innovative alternatives to antibiotics.

Other priorities discussed during the above-mentioned Executive Committee meetings included vaccinology and diagnostics. These issues are currently being addressed by the STAR-IDAZ IRC, but will not be covered in this report, which focuses on diseases/syndromes rather than technologies.

This report provides an overview of the state of the art of research, at a global level, for each of the selected diseases, providing information on:

1. Existing or planned global networks aiming at guiding future research on the topic, and that are acting as STAR-IDAZ IRC Working Groups (see below).
2. Identified research gaps on control tools (diagnostics, vaccines, and pharmaceuticals), extracted from the DISCONTOOLS database.
3. Recent research advances, providing an overview of a selection of highly relevant papers on the subject matter[1].
4. Ongoing research, presenting a non-exhaustive list of ongoing research projects funded by the STAR-IDAZ IRC partners.

For each of its priority diseases/issues, STAR-IDAZ IRC is establishing geographically balanced Working Groups (WGs) of experts to perform gap analyses and to draw research roadmaps on the selected diseases/issues. For diseases/issues where global networks dedicated to gap analyses already exist, these groups were requested to support the STAR-IDAZ IRC and act as WGs. The 'Existing global research networks' sections describe the pre-existing global network or, when this is not present, the STAR-IDAZ IRC newly established WG for each priority disease.

For the selection of articles outlined in the 'Recent research advances' sections, we reviewed the literature published on the priority diseases and selected key articles presenting overviews of the current state of knowledge or providing significant advances in science. Due to the large volume of literature published on the selected diseases/issues, it was not feasible to include a comprehensive list of recent publications, but only a selection of a few highly relevant one, selected by SIRCAH.

The 'Ongoing research' sections present lists of projects targeting the selected priority diseases, classified based on the country of origin and name of the funding body issuing the project. The lists only focus on projects issues by STAR-IDAZ IRC and STAR-IDAZ Network Members, and are non-exhaustive, being based on information extracted from the reports of the STAR-IDAZ IRC Executive meetings, and from Regional Network meetings. Nevertheless, in the view of the authors, such lists still provide a valuable tool to support decision making by research funders, providing support in avoiding duplication of efforts and identifying potential synergies and collaborations.

1. African Swine Fever (ASF)

Global network: Global African Swine Fever Research Alliance (GARA)

The Global African Swine Fever Research Alliance (GARA) was launched with the aim of establishing and sustaining global research partnerships that will generate scientific knowledge and tools to contribute to the prevention, control and, where feasible, eradication of African Swine Fever (ASF).

The GARA has, to date, 38 partners coming from all regions of the world and several stakeholders, including STAR-IDAZ. In 2020, Alex Morrow, Chair of SIRCAH, was elected as Finance Director for the GARA (2020-2023), and joined its Executive Committee.

The GARA objectives are to:

- Identify research opportunities and facilitate collaborations within the Alliance;
- Conduct strategic and multi-disciplinary research to better understand ASF;
- Determine social and economic drivers and impact of ASF;

1 Previous editions of the report contained a selection of scientific papers over 3-years (i.e., the year of the report's publication and the two previous ones). Starting with the current report, it was decided to provide focus on most recent finding only (i.e., occurred over the past year), and to delete reference to older papers.

- Develop novel and improved tools to support the prevention and control of ASF;
- Determine the impact of ASF prevention and control tools; and
- Serve as a communication and technology sharing gateway for the global ASF research community and stakeholders.

GARA Members conducted research gap analyses on ASF diagnostics, vaccinology, epidemiology and virology, which are now periodically updated during the group biannual meetings. These meetings also provide an opportunity for researchers to network and exchange new knowledge about the disease and the development of control tools.

GARA meets periodically every two years to share information about recent scientific advancement on ASF control and update the research gap analysis. The last meeting was held in Cagliari, Italy in 2018, and the next one was planned to take place in 2020 in Kampala, Uganda. Due to the current COVID-19 pandemic, the physical meeting was postponed, and a webinar was held on 24 – 25th August 2020, to provide the opportunity to get up-to-date information on ASF, in terms of geography, diagnostics, sequencing and vaccine development. The meetings' presentations are available at this **link**. The next GARA scientific meeting is planned to take place in August 2021 in Uganda.

SIRCAH had agreed with GARA to host a session during the 2020 physical meeting, to be dedicated to the validation of the STAR-IDAZ IRC roadmaps for ASF (disease control, vaccines, and diagnostic tests). As the meeting was postponed, tentatively to 2021, the SIRCAH's session was also postponed to 2021.

Draft versions of the STAR-IDAZ IRC **roadmaps** are available on the STAR-IDAZ IRC website.

DISCONTOOLS

R&D needs identified for **ASF**:

- Elucidation of the immune response to infection for the identification of target proteins and genes for vaccination.
- Characterisation at genome level of ASFV infection with different isolates.
- Characterisation of the different epidemiological scenarios worldwide for ASF and design ASF control and eradication strategies for each of them.
- Diagnostics: i) expansion of field validation for all tests and appropriate specimens; ii) established cell lines that make virus isolation a cost-effective test for its implementation at the National Reference Laboratories; iii) improvements in molecular characterization tests to determine the source of the outbreaks; and iv) develop DIVA test to allow and accurate monitoring of the effectiveness of the potential vaccine.
- Major efforts to provide an effective and sufficiently effective, safe and DIVA vaccine for wild boar and domestic pigs.

Recent developments

African swine fever – A review of current knowledge (Blome et al., 2020[2])

In this review, published in Virus Research, the authors highlight knowledge gaps and controversial opinions related to African swine fever (ASF), focussing on current knowledge and advances in ASF virology, clinical disease upon infection with recent strains, epidemiology, diagnosis, and control.

2 **Blome, S., Franzke, K., & Beer, M. (2020). African swine fever–A review of current knowledge. Virus Research, 198099.**

Development of a highly effective African swine fever virus vaccine by deletion of the I177L gene results in sterile immunity against the current epidemic Eurasia strain (Borca et al., 2020[3])

In this article, published in Journal of Virology, the authors report the discovery that the deletion of a previously uncharacterised gene, I177L, from the highly virulent African swine fever virus (ASFV) 2007 Georgia isolate (ASFV-G) produces complete virus attenuation in swine. Animals inoculated intramuscularly with the virus lacking the I177L gene, ASFV-G-ΔI177L, at a dose range of 102 to 106 50% haemoadsorbing doses (HAD50), remained clinically normal during the 28-day observational period. All ASFV-G-ΔI177L-infected animals had low viremia titres, showed no virus shedding, and developed a strong virus-specific antibody response; importantly, they were protected when challenged with the virulent parental strain ASFV-G. The authors claim that ASFV-G-ΔI177L is one of the few experimental vaccine candidate virus strains reported to be able to induce protection against the ASFV Georgia isolate, and it is the first vaccine capable of inducing sterile immunity against the current ASFV strain responsible for recent outbreaks.

A Pool of Eight Virally Vectored African Swine Fever Antigens Protect Pigs Against Fatal Disease (Goatley et al., 2020[4])

In an article, published in Vaccines, the authors describe the recent discovery of a pool of viral proteins that appeared to be suitable to be used to produce subunit vaccines for African swine fever (ASF). The described pool appeared to induce both antigen and ASFV-specific antibody and cellular immune responses and to protect 100% of pigs from fatal disease after challenge with a normally lethal dose of virulent ASF virus. In the author's view, this data provides the basis for the further development of a subunit vaccine against ASF.

Could wild boar be the Trans-Siberian transmitter of African swine fever? (Joka et al., 2020[5])

In this article, published in Transboundary and Emerging Diseases, the authors investigate, and prove false, the hypothesis that the introduction of African swine fever (ASF) from Europe to China happened due to natural wild boar movements.

Identification of a Continuously Stable and Commercially Available Cell Line for the Identification of Infectious African Swine Fever Virus in Clinical Samples (Rai et al., 2020[6])

In this article, published in Viruses, the authors report the identification of a commercially available cell line, MA-104, as a suitable substrate for virus isolation of African swine fever virus.

3 **Borca, M.V., Ramirez-Medina, E., Silva, E., Vuono, E., Rai, A., Pruitt, S., Holinka, L.G., Velazquez-Salinas, L., Zhu, J., Gladue, D.P. (2020). Development of a highly effective African swine fever virus vaccine by deletion of the I177L gene results in sterile immunity against the current epidemic Eurasia strain. Journal of Virology, doi: 10.1128/JVI.02017-19**

4 **Goatley, L.C.; Reis, A.L.; Portugal, R.; Goldswain, H.; Shimmon, G.L.; Hargreaves, Z.; Ho, C.-S.; Montoya, M.; Sánchez-Cordón, P.J.; Taylor, G.; Dixon, L.K.; Netherton, C.L. (2020). A Pool of Eight Virally Vectored African Swine Fever Antigens Protect Pigs Against Fatal Disease. Vaccines, 8(2), 234, doi: 10.3390/vaccines8020234**

5 **Joka, F. R., Wang, H., van Gils, H., & Wang, X. Could wild boar be the Trans-Siberian transmitter of African swine fever?. Transboundary and Emerging Diseases.**

6 **Rai, A., Pruitt, S., Ramirez-Medina, E., Vuono, E. A., Silva, E., Velazquez-Salinas, L., Carrillo, C., Borca, M. V., Gladue, D. P. (2020). Identification of a Continuously Stable and Commercially Available Cell Line for the Identification of Infectious African Swine Fever Virus in Clinical Samples. Viruses, 12(8), 820, doi: 10.3390/v12080820**

Ongoing research

Non-exhaustive list of ongoing projects on ASF funded by STAR-IDAZ IRC and STAR-IDAZ Network Members:

- Belgium (FPS):
  - Study of the pathogenesis (role of host receptors) of ASF and innate immune response in ASF virus infected domestic pigs.
- China (CAAS):
  - Develop rapid and cost-effective diagnostic tests including to differentiate ASFV vaccinated animals from non-vaccinated animals.
  - Development of a rapid molecular assay for detection of ASFV DNA - a portable Real-time fluorescent RAA assay with a simple and cheap DNA extraction step has been developed for rapid detection of ASFV DNA.
  - Identity of protective mechanisms operating in immune hosts – in particular the role of the early innate immune responses and the role of cell-mediated immune responses in clearing infections.
- European Commission:
  - VACDIVA (**https://vacdiva.eu/**): developing three safe and effective vaccines for domestic pigs and wild boars, their companion DIVA tests and effective tools for control and eradication strategies in Europe.
  - DEFEND (**https://defend2020.eu/**): tackling the emergence of African swine fever and lumpy skin disease in European livestock.
- France (INRAE):
  - Investigation of virus-host interactions.
- Japan (MAFF):
  - Development of ASF vaccine.
- ILRI:
  - Development of ASF candidate attenuated live vaccines using CRISPR-Cas technology
- Netherlands (MinEZ):
  - Determine the main routes for introduction of ASF into the Dutch pig sector or wild boar population and to gather information to assess the likelihood of introduction via these routes.
- Nigeria:
  - ASF Resist: development of innovative sustainable approaches to prevent spread of ASF – phenotypic typing and identification of genotypic features of pigs that survive ASF outbreaks, and development of community-based biosecurity approaches (EU and Africa Union Funding).
- Spain (INIA):
  - Characterisation of relevant genetic determinants for the development of a vaccine against the virus of the ASF.
  - Strategies of protection against ASF: from basic research to the vaccinal prototype.
- Sweden (Formas):
  - Understanding disease spread in wildlife-ASF in wild boar.
  - Unmasking the mechanisms involved in the protection induced by the live attenuated vaccine BA71DCD2 against ASF.

- UK (Defra):
  - ASF viral survival in experimental settings and the role of fomites.
  - Development and evaluation of non-invasive sampling methodologies in wild suids.
  - Development of adenovirus vectored vaccine.
  - Validation of novel methods for rapid detection of ASF virus and antibodies using LFD.
  - Validation of the use of ZMAC pig macrophage cell line for ASF virus isolation.
  - Identify/develop cell lines that replace primary cultures for improved virus isolation techniques.
- USA (USDA):
  - Development/discovery a stable cell line supporting ASFV replication (DHS).
  - Identity of protective antigens of ASF virus.

2. Bovine tuberculosis (bTB)

Global network: Global Research Alliance for Bovine Tuberculosis (GRAbTB)

The Global Research Alliance for Bovine Tuberculosis (GRAbTB) was initiated under the STAR-IDAZ project, so as to facilitate research cooperation and technical exchange on bovine tuberculosis (bTB).

The GRAbTB has, to date, 15 partners coming from Asia and Australasia, the Americas and Europe, and is looking to expand the network.

The GRAbTB Strategic Goals are to:

Identify research opportunities and facilitate collaborations within the Alliance

- Conduct strategic and multi-disciplinary research to better understand bTB;
- Develop novel and improved tools to control bTB;
- Serve as a communication and technology sharing gateway for the global bTB research community and stakeholders;
- Promote collaboration with the human TB research community.

Over two workshops since 2014, GRAbTB have performed research gap analyses on bTB epidemiology and control, diagnostics, vaccinology and host-pathogen interaction. In 2017, based on these gap analyses, three research roadmaps have been drafted by SIRCAH in collaboration with GRAbTB on bTB vaccines, diagnostics and epidemiology. These roadmaps were discussed by GRAbTB and other bTB experts at a workshop, held in Birmingham, UK in December 2017. After the meeting, SIRCAH and GRAbTB worked on the refining and finalisation of the roadmaps. The structure of generic roadmaps for diagnostic and disease control strategies were further discussed in a meeting of the GRAbTB Executive Committee, that was held in London, UK in July 2019. The discussion led to improvements to the diagnostics roadmap and on the disease control strategies one. GRAbTB will organise subgroups to produce lead summaries for the revised roadmaps for diagnostics and disease control strategies, and to provide a final validation of the vaccine development roadmap, that is published on the STAR-IDAZ IRC website).

The next meeting of the GRAbTB was planned to be held on the side of the 7th International Conference on *Mycobacterium bovis*, that should have been held in Galway, Ireland in June 2020. Due to the COVID-19 situation, the meeting was postponed to June 2021. Thus, the GRAbTB meeting was also postponed to that date.

A draft version of the STAR-IDAZ IRC **roadmap** for the development of bTB vaccines is available on the STAR-IDAZ IRC website.

DISCONTOOLS

R&D needs identified for **bTB**:

- The development of defined **skin test reagents** based on specific *M. bovis* antigens to overcome the limitations of largely undefined and difficult to produce and standardize tuberculins.
- Rapid, specific and simple **diagnostic tests for live animals**, particularly for cattle in developing countries, and for wildlife species.
- Improved delivery systems for the application of **vaccines in wildlife**.
- Further investigations into the **host pathogen interactions** and the immune response to support the development of new vaccines and better diagnostic tools.
- A better understanding of the **epidemiology of *M. bovis*** infections in cattle and cattle herds to enable strategies for the use of new vaccines when available.
- Information on infection by and pathogenesis of *M. bovis*, *M. caprae*, *M. pinnipedii* and even *M. tuberculosis* in other animal species.

Recent developments

Development of a diagnostic compatible BCG vaccine against Bovine tuberculosis (Chandran et al., 2019[7])

This article, published in Scientific Reports, presents research into the development of a synergistic bovine tuberculosis (bTB) vaccine and diagnostic approach that would permit the vaccination of cattle without interfering with the conventional PPD-based surveillance. The aim was to identify antigenic proteins that could be deleted from Bacille Calmette-Guérin (BCG) without affecting the persistence and protective efficacy of the vaccine in cattle, widening the pool of *Mycobacterium bovis* antigens that could be used as targets for Differentiating Infected from Vaccinated Animals (DIVA) tests. The authors identified and inactivated immunogenic but non-essential genes in BCG Danish to create a diagnostic-compatible triple knock-out ΔBCG TK strain. The protective efficacy of the ΔBCG TK was tested in guinea pigs experimentally infected with *M. bovis* by aerosol and found to be equivalent to wild-type BCG. A complementary diagnostic skin test was developed with the antigenic proteins encoded by the deleted genes which did not cross-react in vaccinated or in uninfected guinea pigs.

A defined antigen skin test for the diagnosis of bovine tuberculosis (Srinivasan et al. 2019[8])

An article published in Science Advances describes the development and evaluation of a novel peptide-based defined antigen skin test (DST) to diagnose bovine tuberculosis (bTB) and to differentiate infected from vaccinated animals (DIVA). The results, in laboratory assays and in experimentally or naturally infected animals, demonstrated that the peptide-based DST provides DIVA capability and equal or superior performance over the extant standard tuberculin surveillance test. In the authors' view, the DST presents the potential to considerably improve a century-old standard and to enable the development and implementation of critically needed surveillance and vaccination programs to accelerate bTB control.

7 **Chandran, A., Williams, K., Mendum, T., Stewart, G., Clark, S., Zadi, S., McLeod, N., Williams, A., Villarreal-Ramos, B., Vordermeier, M., Maroudam, V., Prasad, A., Bharti, N., Banerjee, R., Kasibhatla, S. M., McFadden, J. (2019). Development of a diagnostic compatible BCG vaccine against Bovine tuberculosis. Scientific Reports 9, 17791, doi: 10.1038/s41598-019-54108-y**

8 **Srinivasan, S., Jones, G., Veerasami, M., Steinbach, S., Holder, T., Zewude, A., Fromsa, A., Ameni, G., Easterling, L., Bakker, D., Juleff, N., Gifford, G., Hewinson, R.G., Vordermeier, H.M., Kapur, V. (2019). A defined antigen skin test for the diagnosis of bovine tuberculosis. Science Advances 5(7):eaax4899, doi: 10.1126/sciadv.aax4899**

Risk alleles for tuberculosis infection associate with reduced immune reactivity in a wild mammalian host (Tavalire et al. 2019[9])

An article published in Proceedings of the Royal Society B describes the identification of two loci near genes involved in macrophage activation and pathogen degradation that additively increase risk of bovine tuberculosis infection by up to ninefold in wild African buffalo. The authors observed genotype-specific variation in IL-12 production indicative of variation in macrophage activation. Overall, the study findings provide measurable differences in infection resistance at multiple scales by characterising the genetic and inflammatory variation driving patterns of infection in a wild mammal.

Reconsidering Mycobacterium bovis as a proxy for zoonotic tuberculosis: a molecular epidemiological surveillance study (Duffy et al., 2020[10])

In an article, published in the Lancet Microbe, researchers aimed to estimate the human prevalence of animal-associated members of the *Mycobacterium tuberculosis* complex (MTBC) at a large referral hospital in India. The authors successfully identified, using molecular tools (PCR and, where needed, whole-genome sequencing, WGS), species and subspecies of 940 positive mycobacteria growth indicator tube (MGIT) cultures from patients with suspected tuberculosis. The isolates consisted of *M. tuberculosis* (97·1% isolates), *M. orygis* (0·7%), *M. bovis BCG* (0·5%), and non-tuberculous mycobacteria (1·6%). Wild-type *M. bovis* was not identified. The authors concluded that *M. bovis* prevalence in humans is an inadequate proxy of zoonotic tuberculosis. In addition, they suggested that the recovery of *M. orygis* from humans highlights the need to use a broadened definition, including MTBC subspecies such as *M orygis*, to investigate zoonotic tuberculosis. Lastly, the authors conclude that the identification of *M. tuberculosis* in cattle also reinforces the need for One Health investigations in countries with endemic bovine tuberculosis.

Ongoing research

Non-exhaustive list of ongoing projects on bTB funded by STAR-IDAZ IRC and STAR-IDAZ Network Members:

- Argentina (INTA):
  - Comprehensive analysis of protective innate immune response elicited by *Mycobacterium bovis* strains and a vaccine candidate against tuberculosis.
  - Validation of an ELISA test to complement PPD Skin test.
  - Validation of molecular techniques applicable for diagnosis in veterinary laboratories.
- Bill & Melinda Gates Foundation:
  - Validation of BCG efficacy.
- European Commission:
  - bTB-Test (**http://h2020.btb-test.icmpp.ro/index.php**): investigating the feasibility of a non-invasive methodology for the diagnosis of bovine tuberculosis in cattle (detection via volatile organic compounds of the disease fingerprint in several non-invasive samples, employing analytical methods and chemical sensing devices).

9 **Tavalire, H.F., Hoal, E.G., le Roex, N., van Helden, P.D., Ezenwa, V.O., Jolles, A.E. (2019). Risk alleles for tuberculosis infection associate with reduced immune reactivity in a wild mammalian host. Proceedings of the Royal Society B, 286(1907):20190914, doi: 10.1098/rspb.2019.0914**

10 **Duffy, S. C., Srinivasan, S., Schilling, M. A., Stuber, T., Danchuk, S. N., Michael, J. S., Venkatesan, M., Bansal, N., Maan, S., Jindal, N., Chaudhary, D., Dandapat, P., Katani, R., Chothe, S., Veerasami, M., Robbe-Austerman, S., Juleff, N., Kapur, V., Behr, M. A. (2020). Reconsidering Mycobacterium bovis as a proxy for zoonotic tuberculosis: a molecular epidemiological surveillance study. The Lancet Microbe 1(2): e66-e73, doi: 10.1016/S2666-5247(20)30038**0

- Ireland (DAFM):
  - Development of genomic epidemiology systems for tracking and eradicating *Mycobacterium bovis* in Ireland.
  - Targeted genome editing to enhance genetic resistance to *Mycobacterium bovis* infection in domestic cattle populations.
- Italy (MINSAL):
  - Automation and validation of phages test to *detect Mycobacterium avium subsp. paratuberculosis* (Map) in bovine clinical samples.
  - Study on association of polymorphisms (SNP) of Toll Like Receptor (TLR) with the status of the infection *Mycobacterium avium subsp. paratuberculosis* in bovines.
- Netherlands (MinEZ):
  - Development of specific lama VHH antibodies against bTB targets.
  - Identification of B cell epitopes relevant for different disease stages.
  - Peptide epitope discovery.
  - Role of macrophages in clearance and persistent infection.
- UK (Defra):
  - Development of DIVA skin test reagent and replacement of bovine tuberculin international standard.

3. Brucellosis

Global network

Under the STAR-IDAZ project, an expert group on brucellosis was formed in 2014 to conduct a first research gap analysis. Lead summaries for a vaccine roadmap were developed based on these inputs and circulated to that same group of experts for comments in 2018. In order to improve the commitment of the experts in collaborating with the STAR-IDAZ IRC, and to formally establish a STAR-IDAZ IRC Working Group (WG) for brucellosis, SIRCAH representatives participated, giving a presentation, in the 2019 International Brucellosis Society (IBS) Meeting, that was held as a satellite of the Conference of Research Workers in Animal Diseases (CRWAD) meeting in November 2019, in Chicago. The aim of the presentation was to introduce the activities and *modus operandi* of the STAR-IDAZ IRC, and to call for volunteers to revise the draft research roadmap for brucellosis vaccines and to update the research gap analyses for brucellosis diagnostics and disease control. After the meeting, IBS proposed a list of experts to join the STAR-IDAZ WG on brucellosis, and it was agreed that a first meeting of the WG should have been held as a side workshop at the next Global Brucellosis meeting, that was supposed to be held in September 2020 in Italy. As, due to the COVID-19 pandemic, the Global meeting was postponed to 2021, the meeting of the STAR-IDAZ IRC WG will also be postponed, as to hold the two events back-to-back.

A draft version of the STAR-IDAZ IRC **roadmap** for the development of brucellosis vaccines is available on the STAR-IDAZ IRC website.

DISCONTOOLS

R&D needs identified for **Brucellosis**:

- A better understanding of the epidemiology, diagnosis and immunoprophylaxis of brucellosis in less common livestock species (camelids, yaks, water buffaloes, …).
- **Improved vaccines** (more protective, stable, affordable, and less pathogenic), including immunologically tagged vaccines and complementary DIVA tests.
- A better understanding of latent infection in animals.
- **Socio-economic studies** under different situations to prioritize interventions in developing countries.
- **Molecular methods for typing** of Brucella strains.

Recent developments

Development of attenuated live vaccine candidates against swine brucellosis in a non-zoonotic B. suis biovar 2 background (Aragón-Aranda et al., 2020[11])

An article, published in Veterinary research, describes the investigation of the potential use of several *Brucella suis* biovar 2 rough (R) and smooth (S) lipopolysaccharide (LPS) mutants as vaccines for swine brucellosis. Several of the tested mutants proved effective to reduce CFU/spleen in mouse models. As compared to other *B. suis* vaccine candidates described before, the mutants described in this paper simultaneously carry irreversible deletions easy to identify as vaccine markers, lack antibiotic-resistance markers and were obtained in a non-zoonotic background. As all R vaccines interfere in ELISA and other widely used assays, experimental studies in the natural host would be necessary to determine if R mutants vaccines candidates would be advantageous as compared to some of the S ones.

Immunoproteomics of Brucella abortus reveals potential of recombinant antigens for discriminating vaccinated from naturally infected cattle (Faria et al., 2020[12])

In this article, published in Microbial Pathogenesis, the authors report the results of a study aimed at the identification of recombinant *Brucella abortus* antigens to be used for discriminating vaccinated from naturally infected cattle. A bidimensional (2D) immunoblot-based approach was used to find immunogenic proteins to be used in brucellosis serodiagnosis, particularly with ability to be employed in DIVA (Differentiating Infected from Vaccinated Animals) strategy. Immunoproteomic profile of *B. abortus* strain 2308 was analysed in 2D western blotting using pooled sera from S19 vaccinated animals, RB51 vaccinated animals, *B. abortus* naturally infected animals, and non-vaccinated seronegative animals. The evaluation of the antigens differentially immunoreactive against the groups of sera showed three proteins of particular importance: MDH (malate dehydrogenase) immunoreactive for S19-vaccinated animals, SOD (superoxide dismutase) reactive for infected animals, and ABC transporter (multispecies sugar ABC transporter) reactive against sera from vaccinated animals (S19 and RB51). These three proteins were produced in *E. coli* and tested in an indirect ELISA (I-ELISA). In I-ELSIA, comparison between vaccinated animals and seropositive and seronegative animals showed significant differences for MDH, and for SOD sera from non-vaccinated naturally infected animals exhibited significant difference in comparison with all other groups. Overall, these results suggest that the combined use of MDH and SOD could be successful employed in an LPS-free protein based serodiagnosis approach to detect bovine brucellosis and to discriminate vaccinated from naturally infected animals, in early post-vaccination stages.

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11 **Aragón-Aranda, B., de Miguel, M. J., Lázaro-Antón, L., Salvador-Bescós, M., Zúñiga-Ripa, A., Moriyón, I., ... & Conde-Álvarez, R. (2020). Development of attenuated live vaccine candidates against swine brucellosis in a non-zoonotic B. suis biovar 2 background. Veterinary research, 51(1), 1-14.**

12 **Faria, A. R., Dorneles, E. M. S., da Fonseca Pires, S., de Andrade, H. M., & Lage, A. P. (2020). Immunoproteomics of Brucella abortus reveals potential of recombinant antigens for discriminating vaccinated from naturally infected cattle. Microbial Pathogenesis, 104345.**

Vaccine Candidate Brucella melitensis 16MΔvjbR Is Safe in a Pregnant Sheep Model and Confers Protection (Hensel et al., 2020[13])

An article published in mSphere describes the investigation of a vaccine candidate for *Brucella melitensis* in sheep. Aim of the study was to assess if the candidate B*. melitensis* 16MΔvjbR was safer than the currently used *B. melitensis* strain Rev. 1 in pregnant sheep. To evaluate their comparative safety, pregnant sheep (n = 6) were vaccinated subcutaneously with 1 × 1010 CFU/ml of 16MΔvjbR or 1 × 109 CFU/ml Rev. 1 at a highly susceptible stage of gestation (approximately 70 days). 16MΔvjbR resulted in only 1 abortion (1 of 6) compared with 4 of 6 (66.7%) abortions in the Rev. 1 cohort. Vaccination with 16MΔvjbR resulted in less vertical transmission than Rev. 1 (assessed by culture and histopathology of fetal tissues, to determine if vaccination prevented infection of the foetus). To determine if vaccination was efficacious and could reduce tissue colonization in sheep, the same cohorts of sheep were challenged 5 weeks postpartum by conjunctival inoculation with 1 × 107 CFU/ml *B. melitensis*. Protection was similar between Rev. 1 and 16MΔvjbR, with no statistical difference in colonisation in the target organs. The authors concluded that the 16MΔvjbR vaccine is safer than Rev. 1 for use during pregnancy and provided a similar level of protection as Rev. 1, and that it could be considered an improved candidate for future vaccine trials.

Ongoing research

No ongoing projects on brucellosis have been reported from STAR-IDAZ IRC and STAR-IDAZ Network Members during their periodic meetings in 2020.

4. Foot-and-mouth disease (FMD)

Global network: Global Foot-and-Mouth Research Alliance (GFRA)

The Global Foot-and-Mouth Research Alliance (GFRA) was launched in 2003 with the aim of establishing and sustaining global research partnerships to generate scientific knowledge and discover the tools to successfully prevent, control, and eradicate FMD.

The GFRA has, to date, 27 partners coming from all regions of the world and many stakeholders, including STAR-IDAZ.

The GFRA objectives are to:

- Facilitate research collaborations and serve as a communication gateway for the global FMD research community;
- Conduct strategic research to better understand FMD;
- Development of the next generation of control measures and strategies for their application;
- Determine social and economic impacts of the new generation of improved FMD control; and
- Provide evidence to inform development of policies for safe trade of animals and animal products in FMD-endemic areas.

The GFRA Members conducted research gap analyses on FMD diagnostics, vaccinology, epidemiology biotherapeutics and disinfectants, immunology, and pathogenesis and molecular biology. These are now periodically updated during the group biannual meetings. These meetings also provide an opportunity for researchers to network and exchange new knowledge about the disease and the development of

13 **Hensel, M. E., Garcia-Gonzalez, D. G., Chaki, S. P., Hartwig, A., Gordy, P. W., Bowen, R., ... & Arenas-Gamboa, A. M. (2020). Vaccine Candidate Brucella melitensis 16MΔvjbR Is Safe in a Pregnant Sheep Model and Confers Protection. Msphere, 5(3).**

control tools.

In 2016, the GFRA published the outcomes of its latest gap analyses (2010) in a series of seven scientific papers, which appeared in the *Transboundary and Emerging Diseases* journal in 2016. These will be presented in the section 'Recent developments' of this chapter.

The last GFRA scientific meeting was held in Seoul, Republic of Korea in October 2017, with the aim to bring research scientists from all over the world together to discuss their work and progresses on science and innovation for FMD control and response.

A meeting for updating the 2010 GFRA research gap analyses was held in Buenos Aires, Argentina, in June 2018. The purpose of the meeting was to bring together FMD experts worldwide to analyse and discuss vacant areas and pending challenges in relation to the control of the disease on a global scale. The revised document will be published on the GFRA website as soon as finalised. The meeting also served as a basis for developing STAR-IDAZ IRC FMD research roadmaps. Three draft roadmaps (one on diagnostics, one on vaccines and one on disease control strategies) were drafted by SIRCAH and, after having been revised by the STAR-IDAZ IRC Scientific Committee, were sent to GFRA experts' groups. In October 2019, SIRCAH organised a satellite workshop on the side of the GFRA Scientific Meeting held in Bangkok, Thailand, to get final expert validation of the roadmaps.. About 30 experts participated in the workshop, bringing a well-balanced range of specialisms (across diagnostics, vaccines, and epidemiology) and wide geographical representation. The experts identified the main priority leads for each of the roadmaps, and looked into the details of the identified challenges for each of these leads. The roadmaps were updated based on the received inputs, and the final version was circulated after the meeting to obtain endorsement from the group.

The validated versions of the STAR-IDAZ IRC **roadmaps** are available on the STAR-IDAZ IRC website.

DISCONTOOLS

R&D needs for **FMD**:

- Faster diagnostics and sensitive pen side tests along with the development of more effective and specific tests for differentiating between antibodies due to infection and vaccination.
- Sufficient panels for test validation across all serotypes and species.
- Knowledge about virus transmission and persistence in vaccinated populations and reliability of tests to differentiate vaccinated from infected animals.
- Support for fundamental immunology and for animal studies.
- Knowledge on circulating isolates in endemic regions for selecting the vaccine antigens in endemic settings.
- Better serological predictors of protection afforded by vaccination

Recent developments

Foot-and-Mouth Disease surveillance using pooled milk on a large-scale dairy farm in an endemic setting (Armson et al., 2020[14])

In this paper, published in Frontiers in Veterinary Science, the authors evaluated pooled milk for foot-and-mouth disease virus (FMDV) surveillance on a large-scale dairy farm that experienced two FMD outbreaks, despite regular vaccination and strict biosecurity practices. During the study, FMDV RNA was

14 **Armson, B., Gubbins, S., Mioulet, V., Qasim, I.A., King, D.P., Lyons, N.A. (2020) Foot-and-Mouth Disease Surveillance Using Pooled Milk on a Large-Scale Dairy Farm in an Endemic Setting. Frontiers in Veterinary Science, 7:264, doi: 10.3389/fvets.2020.00264**

detected in 42 (5.7%) of the 732 pooled milk samples. The FMDV positive milk samples were temporally clustered around reports of new clinical cases, but with a wider distribution. For further investigation, a model was established to predict real-time RT-PCR (rRT-PCR) CT values: this explained some of the instances where there were positive results by rRT-PCR, but no new clinical cases and suggested that subclinical infection occurred during the study period. In the authors' view, the results from this pilot study indicate that testing pooled milk by rRT-PCR may be valuable for FMD surveillance and has provided evidence of subclinical virus infection in vaccinated herds that could be important in the epidemiology of FMD in endemic countries where vaccination is used.

Detection of bovine antibodies against a conserved capsid epitope as the basis of a novel universal serological test for foot-and-mouth disease (Asfor et al., 2020[15])

In this paper, published in the Journal of Clinical Microbiology, the authors present their work on the development of a universal test for the detection of antibodies against foot-and-mouth disease virus (FMDV) structural proteins (SP). As, to date, SP tests need to be tailored to the individual FMD virus serotype and their sensitivity performances may be affected by antigenic variability within each serotype and mismatching between tests reagents, a universal test would simplify frontline diagnostics and facilitate large-scale serological surveillance and post-vaccination monitoring. The researchers characterised a highly conserved region in the N terminus of FMDV capsid protein VP2 (VP2N) using a panel of intertypic-reactive monoclonal antibodies, revealing a universal epitope in VP2N which could be used as a peptide antigen to detect FMDV-specific antibodies against all types of the virus. A VP2-peptide ELISA (VP2-ELISA) was optimised using experimental and reference antisera from immunised, convalescent and negative animals. The developed universal VP2-ELISA provided sensitive (99 %) and specific (93%) detection of antibodies to all FMDV strains used in this study.

Quantifying the transmission of Foot-and-Mouth Disease virus in cattle via a contaminated environment (Colenutt et al., 2020[16])

An article, published in mBio, present the quantification of indirect transmission of foot-and-mouth disease virus (FMDV) via a contaminated environment. The authors carried out a series of transmission experiments to determine the dose-response relationship between environmental contamination and transmission of FMDV in cattle from measurements of viral shedding and rates of environmental contamination and survival. Seven out of ten indirect exposures resulted in successful transmission. The basic reproduction number for environmental transmission of FMDV in the experimental setting was estimated at 1.65, indicating that environmental transmission alone could sustain an outbreak. Importantly, detection of virus in the environment prior to the appearance of clinical signs in infected cattle and successful transmission from these environments highlights there is a risk of environmental transmission even before foot-and-mouth disease (FMD) is clinically apparent in cattle. Estimated viral decay rates suggest that FMDV remained viable in this environment for up to 14 days, emphasising the requirement for stringent biosecurity procedures following outbreaks of FMD and the design of control measures that reflect the biology of a pathogen.

15 **Asfor, A., Howe, N., Grazioli, S., Berryman, S., Parekh, K., Wilsden, G., Ludi, A., King, D.P., Parida, S., Brocchi, E., Tuthill, T.J. (2020). Detection of bovine antibodies against a conserved capsid epitope as the basis of a novel universal serological test for foot-and-mouth disease. Journal of Clinical Microbiology, online 18 March 2020, doi: 10.1128/JCM.01527-19**

16 **Colenutt, C., Brown, E., Nelson, N., Paton, D. J., Eblé, P., Dekker, A., Gonzales, J. L., Gubbins, S. (2020). Quantifying the Transmission of Foot-and-Mouth Disease Virus in Cattle via a Contaminated Environment. mBio, 11(4), e00381-20, doi: 10.1128/mBio.00381-20**

Pervasive within-host recombination and epistasis as major determinants of the molecular evolution of the Foot-and-mouth disease virus capsid (Ferretti et al., 2020[17])

In this paper, published in PLoS Pathogens, the authors investigated the recombination rates of foot-and-mouth disease virus (FMDV) in African buffaloes in an experimental setting. Animals were inoculated with a SAT-1 FMDV strain containing two major viral sub-populations differing in their capsid sequence, as to enable the detection of extensive within-host recombination in the genomic region coding for structural proteins and to allow recombination rates between the two sub-populations to be estimated. The study revealed that during FMDV co-infections by closely related strains, capsid-coding genes recombine within the host at a much higher rate than expected, despite the presence of strong constraints dictated by the capsid structure. In the view of the authors, these findings support a major role for recombination and epistasis in the intra-host evolution of FMDV.

Ongoing research

Non-exhaustive list of ongoing projects on FMD funded by STAR-IDAZ IRC and STAR-IDAZ Network Members:

- Argentina (INTA):
  - Assessment of the immunogenicity of natural empty capsids and assessment of novel candidates in the mouse model.
  - Characterisation by NGS sequencing of the whole sample bank of the 2000-2001 epidemic in Argentina.
  - Defining cross-protecting epitopes by using chimeric recombinant FMDV particles (INTA-USDA collaboration).
  - Defining different immunisation schedules and antigen combination to induce broader immune responses.
  - Development of a site A/1 less infectious clone to evaluate a wider cross-protective response than the complete virus. Methodology optimization for sequence and analysis of Cell B activated clones.
  - Development of FMDV capsids containing non-replicative RNA / Study of viral dynamics by NGS to identify virulence factors (mouse model).
  - FAL ELISA application in formulated vaccines (ThermoFisher grant).
  - Identifying cross-reactive epitopes/paratopes by screening scFv libraries (IVVN Pump Priming Grant).
  - Validation of avidity and isotype ELISAs (EU-FMD FAR grant).
- Australia (FMD Ready Project):
  - Apply a sequence-based approach to estimate the true prevalence of different lineages.
  - Assess costs-benefits of FMD control strategies.
  - Assess the direct and indirect cost, and control cost of FMD outbreaks in different contexts.
  - In vitro quality control methods (reduce animal use, at least to reduce number of batches tested, also including ruminants).
  - Isolation of viruses from Lateral Flow Devices.
  - Serologically differentiation among cross-reacting serotypes.

17 **Ferretti, L., Pérez-Martín, E., Zhang, F., Maree, F., de Klerk-Lorist, L.M., van Schalkwykc, L., Juleff, N.D., Charleston, B., Ribeca, P. (2020). Pervasive within-host recombination and epistasis as major determinants of the molecular evolution of the Foot-and-mouth disease virus capsid. PLoS Pathogens 16(1):e1008235. doi: 10.1371/journal.ppat.1008235**

- Bill & Melinda Gates Foundation:
  - Define the roles of non-neutralising antibody-bound virus complexes with immune cells in vivo in target species.
  - Determine what factors define broad protection and broad coverage, through identifying cross-protecting antigens/epitopes.
  - Development of a cross protective/multivalent DNA/RNA vaccine.
  - In vitro quality control methods (reduce animal use, at least to reduce number of batches tested, also including ruminants).
- China (CAAS):
  - Cooperative creation and application studies of new products for prevention and control of major transboundary animal diseases (FMD and PPR).
  - Defining the mechanism of host range variation of FMDV: identifying the determinant of viral pathogenicity and host range.
  - Development and validation of Rapid New VNT assay.
  - Development of a GeXP Analyzer-Based Multiplex PCR Assay for FMDV characterization: Optimization of GeXP assay for FMDV SAT2 serotyping.
  - Development of an alternative method for efficacy testing of FMD vaccine: development of ELISA or 146s antigen quantitative method as an alternative method for efficacy testing of FMD vaccine.
  - Development of an isothermal amplification assay for rapid detection FMDV and other viruses in ruminants.
  - Development of FMDV virus-like particles against different serotype.
  - Development of novel diagnostic test technology against critical diseases affecting sheep and cow (including FMD).
  - Development of rapid and long-acting inactivated FMD vaccine.
  - Identifying cross-protecting antigens of FMD.
  - Immune and cellular mechanisms related to outcome of FMDV infection. We are currently working on immunomodulatory factors (including STING and RPIK3) in controlling FMDV infection.
  - Screening effector molecules involved in innate and adaptive immunity as adjuvants to improve the efficacy and safety of current inactivated FMD vaccines.
  - Screening non-structural proteins/epitopes of FMD virus inducing strong innate or cellular immune response in pigs.
  - Synthesise mesoporous silica nanoparticles ass adjuvant of FMD vaccine to induce persistent immune response.
- Italy (MINSAL):
  - Replication dynamics of foot-and-mouth disease virus during co-infection in vitro with different strains / serotypes and recovery of infectious aphthous virus from RNA.
- Netherlands (MinEZ):
  - Define in vitro homologous and heterologous correlates of protection.
  - Replacing FMDV challenge tests for VLP vaccines by in-vitro analysis.

- Spain (INIA):
  - Development and optimization of new vaccines and antiviral strategies. the foot and mouth disease virus as a model.
  - Mono and multivalent peptide vaccine constructions against FMD virus: optimised production and studies of structure, stability and biodistribution.
- Uganda:
  - Vaccine matching studies for Uganda.
- UK (Defra):
  - Development and validation of new diagnostic tools to equip the UK National Reference Laboratory for FMD.
  - Reducing the impact of FMD outbreaks through early detection and vaccination.
  - Reducing the impact of foot-and-mouth disease outbreaks through early detection and vaccination (preclinical detection of FMDV in the field).
  - Understanding environmental and airborne transmission to reduce FMD.
  - Use of FMDV sequence data to trace outbreaks and monitor disease incursion risks to the UK (development of analytical tools to reconstruct disease transmission networks at the farm-level).
- USA (USDA):
  - Developing Immunoassays for Determining the Immune Mechanisms Affecting Protective Immunity against FMDV
  - Development of FMD-LL3B3D vaccine platform and 3ABC and 3D companion DIVA diagnostic tests.
  - Development of LAV FMD vaccines using codon deoptimization technology. (b) LAV vaccines strains based on leader gene modifications (c) LAV vaccines based on other genomic modifications.
  - Field studies on FMD ecology in Cameroon, Nigeria, Kenya and Uganda.
  - Inter-species animal to animal transmission as well as feed transmission (various research projects).
  - Investigating cross protective immunity using mosaic FMD vaccines
  - Using peptide arrays to identify relevant FMDV cross protective antigens.

5. Helminths

Global network: Livestock Helminth Research Alliance (LiHRA)

The **Livestock Helminth Research Alliance** (LiHRA) was founded in December 2014, comprising of international partners with a recognised expertise in different disciplines applied to livestock helminth research. LiHRA unites diverse areas of expertise in the field of helminth infections of livestock, and aims to:

- Stimulate collaborative research by enabling exchange of ideas and mobility of young researchers;
- Initiate and coordinate research initiatives at the international and national level;
- Facilitate knowledge exchange with the livestock industry and other stakeholders to respond to their needs;
- Respond to global changes that impact on livestock, farming practices and helminth infections and identify areas for future research;
- Foster technology exchange and standardisation of diagnostic procedures, clinical trial and monitoring approaches throughout Europe.

Through collaboration, LiHRA aims to become the leading research alliance in the field of livestock helminth infections with a mission to develop sustainable helminth control strategies and promote their implementation by the livestock industry. LiHRA has 24 member organisations from 14 European countries and 6 associated organisations from New Zealand, Argentina, USA, Mexico and Canada. LiHRA has had five successful meetings so far, the most recent being 26-27 August 2019 in Ghent (Belgium). In these meetings, members present overviews of their current research areas and discuss pathways for collaboration or new ideas to be explored. LiHRA coordinated responses to 7 international grant calls in the last 4 years. Together with the COST Action **COMBAR**, LiHRA coordinated the development of the STAR-IDAZ helminth research road maps and produced a **summary document** highlighting the key research needs for control of helminth infectious diseases in ruminants.

DISCONTOOLS

R&D needs for **nematodes**:

- Development and implementation of **holistic control strategies** using improved diagnostics, host genetics, nutrition and pasture management to reduce the reliance upon anthelmintics and the threat of anthelmintic resistance.
- Easy-to-use **diagnostics** to identify those animals requiring treatment and tests for early detection of anthelmintic resistance.
- **Anthelmintics with new mode of action** than currently available.
- Development of **complementary control measures**: vaccines, bio-active forages, nutraceuticals.

R&D needs for **liver fluke**:

- More information about how the predicted effects of **climate and environmental change** are influencing the survival and development of the environmental stages of the parasite.
- An understanding of the **immune responses** to fluke (innate and adaptive; protective and suppressive) in naturally exposed ruminants.
- **Genome mapping** to: aid in identification of drug resistant isolates, improving our understanding of drug resistance to different flukicides; develop tools for diagnosis; and differentiating between species and identifying hybrid species.
- Pen-side tests, herd level tests to identify heavily infected beef herds, tests for diagnosis for acute infection or pre-patent infections.

- **Drugs** that are effective against the young immature stages of the parasite.
- **Vaccines** targeting all stages and suitable for any host species.
- Good control programmes no longer reliant on the exclusive use of anthelmintic prophylaxis to address the problems with drug resistance.

R&D needs for *Taenia solium* **cysticercosis**:

- Better knowledge of the **distribution of infection** and delineation of areas of high prevalence, particularly of neurocysticercosis.
- Further information on the effectiveness and **cost-benefit** of (alternative) control/elimination strategies of the infection in humans and pigs in different epidemiological settings.
- Studies on *T. solium* **egg survival**.
- Availability of **simple tests** to detect *T. solium* infections in humans, of pen-side diagnostic tests for individual pigs and for detection of infected carcasses in the abattoir.
- A serological test which is able to **detect living cysts** in the brain.
- A **serum bank** with well documented serum and cerebrospinal fluid samples to study sensitivity, specificity, reproducibility of serological tests.

R&D needs for *Echinococcus:*

- Harmonised **reporting systems** for AE and CE in humans and animals.
- Standardization of validated **molecular tools** for the detection of *Echinococcus spp.* eggs for assessing the degree of contamination of matrices, water and food
- Well-designed, integrated, long lasting **CE control programmes** based on deworming of dogs, vaccination of lambs and culling of old intermediate hosts
- Improved specific and sensitive **fast tests** for the diagnosis and monitoring of control programmes of CE in livestock and intestinal infections in dogs
- European manufacture and registration of the **EG95 vaccine** for livestock and political will and funding to undertake control programmes.

Recent developments

Refugia and anthelmintic resistance: concepts and challenges (Hodgkinson et al., 2019[18])

In this paper Hodgkinson and colleagues scrutinise the role of refugia to manage the problem of anthelmintic resistance. In order to alleviate the selection pressure for resistance and maintain drug efficacy, management strategies increasingly aim to preserve a proportion of the parasite population in 'refugia', unexposed to treatment. While persuasive in its logic, and widely advocated as best practice, evidence for the ability of refugia-based approaches to slow the development of drug resistance in parasitic helminths is currently limited. Moreover, the conditions needed for refugia to work, or how transferable those are between parasite-host systems, are not known. The authors concluded that factors potentially important to refugia, such as the fitness cost of drug resistance, the degree of mixing between parasite sub-populations selected through treatment or not, and the impact of parasite life-history, genetics and environment on the population dynamics of resistance, vary widely between systems. The success of attempts to generate refugia to limit anthelmintic drug resistance are therefore likely to be highly dependent on the system in hand. They recommend research towards the underlying

18 **Hodgkinson, J.E., Kaplan R.M., Kenyon F., Morgan, E.R., Park, A.W, …, Devaney, E. (2019). Refugia and anthelmintic resistance: concepts and challenges.** *International Journal for Parasitology Drugs and Drugs Resistance* **10, 51-57.**

principles of refugia for its application across systems, as well as empirical studies within systems that prove and optimise its usefulness.

Comparative genomics of the major parasitic worms (International Helminth Genomes Consortium, 2019[19])

Parasitic nematodes (roundworms) and platyhelminths (flatworms) cause debilitating chronic infections of humans and animals. The authors report in Nature Genetics on a broad comparative study of 81 genomes of parasitic and non-parasitic worms. They have identified gene family births and hundreds of expanded gene families at key nodes in the phylogeny that are relevant to parasitism. Examples include gene families that modulate host immune responses, enable parasite migration though host tissues or allow the parasite to feed. They reveal extensive lineage-specific differences in core metabolism and protein families historically targeted for drug development. From an in-silico screen, they identified and prioritized new potential drug targets and compounds for testing. This comparative genomics resource will boost progress on the understanding and the combat against parasitic worms.

Initial assessment of the economic burden of major parasitic helminth infections to the ruminant livestock industry in Europe (Charlier et al., 2020[20])

In the framework of the COST Action COMBAR, the authors report a European wide assessment of the economic burden of gastrointestinal nematodes, *Fasciola hepatica* (common liver fluke) and *Dictyocaulus viviparus* (bovine lungworm) infections to the ruminant livestock industry. The economic impact of these parasitic helminth infections was estimated by a deterministic spreadsheet model as a function of the proportion of the ruminant population exposed to grazing, the infection frequency and intensity, the effect of the infection on animal productivity and mortality and anthelmintic treatment costs. In addition, the first estimates are provided on the costs of anthelmintic resistant nematode infections and public research budgets addressing helminth infections in ruminant livestock. The combined annual cost [low estimate-high estimate] of the three helminth infections in 18 participating countries was estimated at € 1.8 billion [€ 1.0–2.7 billion]. Eighty-one percent of this cost was due to lost production and 19 % was attributed to treatment costs. The cost of gastrointestinal nematode infections with resistance against macrocyclic lactones was estimated to be € 38 million [€ 11–87 million] annually. The annual estimated costs are also provided by parasite and by production sector. The authors emphasize that data gaps were present in all phases of the calculations which lead to large uncertainties around the estimates. Accessibility of more granular animal population datasets at EU level, deeper knowledge of the effects of infection on production, levels of infection and livestock grazing exposure across Europe would make the largest contribution to improved burden assessments. The known current public investment in research on helminth control was 0.15 % of the estimated annual costs for the considered parasitic diseases. The authors conclude that the costs of enzootic are similar or higher than reported costs of epizootic diseases and recommend using the data to support decision making in research and policy to mitigate the negative impacts of helminth infections and anthelmintic resistance in Europe.

Antiparasitics in Animal Health: Quo Vadis? (Selzer et a., 2020[21])

In a review, published in Trends in Parasitology, the authors present a nice overview of the current landscape of antiparasitic drugs development, discussing major drivers of future innovation and new approaches to antiparasitic development, and exploring the potential for parasite vaccines.

19 **International Helminth Genomes Consortium (2019). Comparative genomics of the major parasitic worms.** *Nature Genomics* **51, 163-174.**

20 **Charlier, J., Rinaldi, L., Musella, V., Ploeger, H.W., Chartier, C., …, Claerebout, E. (2020). Initial assessment of the economic burden of major parasitic helminth infections to the ruminant livestock industry in Europe.** *Preventive Veterinary Medicine* **182, 105103**

21 **Selzer, P. M., & Epe, C. (2021). Antiparasitics in Animal Health: Quo Vadis?. Trends in Parasitology 37(1),P77-89.**

Ongoing research

Non-exhaustive list of ongoing projects on helminths funded by STAR-IDAZ IRC and STAR-IDAZ Network Members:

- China (CAAS):
  - Compare differences in humoral immune response among sheep, cattle, pigs, mice and rabbits against EG95 vaccine.
  - Development of locally adapted worm control practices in Qinghai-Tibet plateau.
  - Development of novel biological agents for control of echinococcosis.
  - Development of polyvalent vaccines against Echinococcus infections (such as *E. granulosus* and *E. canadensis.*) and Taenia infections (such as *T. multiceps*) in sheep.
  - Genomics research on several Echinococcus species: de novo assembly and genome annotation of *Echinococcus granulosus*, *E. multilocularis*, *E. shiquicus* and others species using PacBio, stLFR and Hi-C assisted assembly technology and comparative genomics and selection of drugs against Echinococcus larva based genomic-assisted drug discovery.
  - Investigation on exosome derived from tapeworms and interaction between host and parasite mediated by exosomes, functional genomics and development of diagnostic reagents/ techniques and vaccines.
  - Investigation on other adjuvants replacing Quil A.
  - Investigation of the roles of *Echinococcosis multilocularis* miRNAs in the regulation of the intermediate host immune response, focusing on miRNA molecules as targets to pinpoint their roles in the host-parasite interplay, which will shed light on the development of novel anti-echinococcosis vaccines and therapies.
  - Modulation of host (cattle and sheep) "omics" and immune responses induced by *Fasciola spp* infection.
  - Yeast expression system is being explored to produce EG95 and TM18 with N-glucosylation(s).
- Spain (INIA):
  - "Your resources are my resources": parasite migration and the fibrinolytic system of the host in fasciolosis.
  - Immunological, genetic and molecular approaches for the control of fasciolosis in ruminants.
- Sweden (Formas):
  - Biologic control of equine strongyles using a predatory fungus (*Duddingtonia flagrans*).
  - Improved diagnostics and control of *Ascaridia galli* infection in Swedish Laying hens.
  - New tools for molecular species identification of *Trichinella spp*.
  - Pasture hygiene methods as a complement to anthelmintic treatment in horse farms.

6. Porcine Reproductive and Respiratory Syndrome (PRRS)

Global network

Under the STAR-IDAZ project, an expert group was formed on Porcine Reproductive and Respiratory Syndrome (PRRS) and, in 2013, this group conducted the first research gap analysis. No formal Working Group (WG) has since been established. SIRCAH drafted a research roadmap for PRRS vaccines and organised a meeting with the above-mentioned experts on the side of the 2018's Conference of Research Workers in Animal Disease (CRWAD) to validate it. The experts validated the **roadmap**, that was published on the STAR-IDAZ IRC website. SIRCAH will start working on the development of roadmaps for diagnostic development and control strategies for PRRS, that will need then to be submitted to the WG for validation.

DISCONTOOLS

R&D needs for **PRRS**:

- **Whole genome analysis** to obtain correct genetic trees as a basis for epidemiological studies (evolution) and to identify the parts of the genome that are linked to the ability to spread, pathogenicity, virulence, immune evasion and immunogenicity.
- **Continuous validation of diagnostics** with the appearance of new PRRS virus isolates. It is important to monitor the genetic sequences of new viruses to ensure that they are detected in the existing PCR/ELISAs. To achieve this, a pan-European PRRS database should be created that would allow simultaneous comparison of PRRS isolates representing most countries in Europe.
- **New generation vaccines** that provide universal protection and that allow differentiating vaccinated animals from infected ones (DIVA). To achieve this, new approaches to vaccine production should be considered, such as multivalent vaccines or subunit vaccines.

Recent developments

Predicting vaccine effectiveness in livestock populations: A theoretical framework applied to PRRS virus infections in pigs (Bitsouni et al., 2019[22])

In this article, published in PLoS ONE, the authors present a theoretical framework for modelling the epidemiological consequences of vaccination with imperfect vaccines of various types, administered using different strategies to herds with different replacement rates and heterogeneity in vaccine responsiveness. Applying the model to the Porcine Reproductive and Respiratory Syndrome (PRRS), the authors examined the influence of these diverse factors alone and in combination, on within-herd virus transmission, and derived threshold conditions for preventing infection in the case of imperfect vaccines inducing limited sterilising immunity. The model developed in this study has practical implications for the development of vaccines and vaccination programmes in livestock populations not only for PRRS, but also for other viral infections primarily transmitted by direct contact.

22 **Bitsouni, V., Lycett, S., Opriessnig, T., & Doeschl-Wilson, A. (2019). Predicting vaccine effectiveness in livestock populations: A theoretical framework applied to PRRS virus infections in pigs. PloS one, 14(8), e0220738.**

New insights about vaccine effectiveness: Impact of attenuated PRRS-strain vaccination on heterologous strain transmission (Chase-Topping et al., 2020[23])

This paper, published in Vaccine, investigated the impact of attenuated Porcine Reproductive and Respiratory Syndrome (PRRS)-strain vaccination on heterologous strain transmission. Two successive transmission trials were performed involving 52 pigs to evaluate the effectiveness of a PRRS vaccinal strain candidate against horizontal transmission of a virulent heterologous strain. PRRS virus was observed in serum and nasal secretions for all but one pig, indicating that vaccination did not protect pigs from becoming infected and shedding the heterologous strain. However, vaccination delayed the onset of viraemia, reduced the duration of shedding and significantly decreased viral load throughout infection. Serum antibody profiles indicated that 4 out of 13 (31%) vaccinates in one trial had no serological response (NSR). A Bayesian epidemiological model was fitted to the data to assess the impact of vaccination and presence of NSRs on PRRS virus transmission dynamics. Despite little evidence for reduction in the transmission rate, vaccinated animals were on average slower to become infectious, experienced a shorter infectious period and recovered faster. Model selection suggests that transmission parameters of vaccinated pigs with NSR were more similar to those of unvaccinated animals. The presence of NSRs in a population, however, seemed to affect the transmission dynamics only marginally. The authors concluded that even when vaccination cannot prevent infection, it can still have beneficial impacts on the transmission dynamics and contribute to reducing a herd's R0.

Porcine reproductive and respiratory syndrome virus infection induces endoplasmic reticulum stress, facilitates virus replication, and contributes to autophagy and apoptosis (Chen et al., 2020[24])

A paper, published in Scientific Reports, presents new insights on the host response to endoplasmic reticulum (ER) stress caused by porcine reproductive and respiratory syndrome (PRRS). It is known that, to manage abnormal ER stress (often observed during viral infection, due to the high amounts of viral proteins being synthesised by the host cell), mammalian cells trigger a response called the unfolded protein response (UPR). PRRSV infection can induce ER stress and activate UPR, but its activation pathways and biological significance requires further investigation. In this study, the authors revealed the associations of ER stress, autophagy, and apoptosis during PRRSV infection, helping an improved understanding of how PRRSV interacts with host cells.

Small molecules block the interaction between porcine reproductive and respiratory syndrome virus and CD163 receptor and the infection of pig cells (Huang et al., 2020[25])

In this paper, published in the Virology Journal, Huang and colleagues aimed to investigate a novel strategy to potentially prevent porcine reproductive and respiratory syndrome virus (PRRS) infection in pigs by blocking the interaction between PRRSV and a key receptor for PRRSV infection (CD163), using small molecules. The authors obtained, through artificial intelligence molecular screening, a set of small molecule compounds predicted to target the scavenger receptor cysteine-rich domain 5 (SRCR5) of CD163. These compounds were screened using a cell-based bimolecular fluorescence complementation (BiFC) assay, and the function of positive hit was further evaluated and validated by PRRSV-infection

23 **Chase-Topping, M., Xie, J., Pooley, C., Trus, I., Bonckaert, C., Rediger, K., ... & Gueguen, S. (2020). New insights about vaccine effectiveness: Impact of attenuated PRRS-strain vaccination on heterologous strain transmission. Vaccine**.

24 **Chen, Q., Men, Y., Wang, D., Xu, D., Liu, S., Xiao, S., & Fang, L. (2020). Porcine reproductive and respiratory syndrome virus infection induces endoplasmic reticulum stress, facilitates virus replication, and contributes to autophagy and apoptosis. Scientific reports, 10(1), 1-13**.

25 **Huang, C., Bernard, D., Zhu, J., Dash, R. C., Chu, A., Knupp, A., Hakey, A., Hadden, M. K., Garmendia, A., Tang, Y. (2020). Small molecules block the interaction between porcine reproductive and respiratory syndrome virus and CD163 receptor and the infection of pig cells. Virology Journal, 17(1), 116, doi: 10.1186/s12985-020-01361-7**

assay using porcine alveolar macrophages (PAMs). The BiFC assay allowed to identify one compound with previously unverified function, 4-Fluoro-2-methyl-N-[3-(3-morpholin-4-ylsulfonylanilino)quinoxalin-2-yl] benzenesulfonamide (designated here as B7), that significantly inhibited the interaction between the PRRSV glycoprotein and the CD163-SRCR5 domain. In addition, the study demonstrated that compound B7 inhibited PRRSV infection of PAMs in a dose-dependent manner, for both type I and type II strains.

Characterization of the IgA response to PRRS virus in pig oral fluids (Ruggeri et al., 2020[26])

A study, published in PLoS ONE, reports the characterisation of the antibody response to porcine reproductive and respiratory syndrome virus (PRRSV) in pig oral fluids (OF). An association between PRRSV-specific IgA responses in OF of gilts and block of PRRSV spread has been reported earlier. Here, the authors investigated *in vitro* the inhibition of PRRSV replication by OF samples with different titres of PRRSV-specific IgA and IgG antibody, using Real-time RT PCR. PRRSV yield reduction in monocyte-derived macrophages was associated with the IgA content in OF samples, whereas the IgG-rich samples were sometimes associated with antibody-dependent enhancement of replication. The study results point at a role of mucosal IgA in the control of PRRSV replication by extra- and/or intracellular interaction with PRRSV, as well as by induction of signals leading to a reduced susceptibility of macrophages to PRRSV infection.

Ongoing research

Non-exhaustive list of ongoing projects on PRRS funded by STAR-IDAZ IRC and STAR-IDAZ Network Members:

- China (CAAS):
  - Establishment of qPCR for simultaneous diagnosis of genotype 1 and 2 PRRSV and investigating its molecular epidemiology.
- Italy (MINSAL):
  - Assessment of the effect of polymorphisms of host genes on resistance to type I PRRSV infection as an alternative system for the control of PRRS.
- Hungary (NAK):
  - Investigation of the genetic diversity of the Hungarian PRRSV strains and the effect of RdRp on their evolution.
- Spain (INIA):
  - Micro-epidemiology of precision of PRRS infection in epidemic farms.

26 **Ruggeri, J., Ferlazzo, G., Boniotti, M. B., Capucci, L., Guarneri, F., Barbieri, I., ... & Amadori, M. (2020). Characterization of the IgA response to PRRS virus in pig oral fluids. PloS one, 15(3), e0229065.**

7. Coronaviruses

Global network

The coronaviruses (CoVs) of interest for the STAR-IDAZ IRC are infectious bronchitis virus (IBV), Middle East Respiratory Syndrome CoV (MERS-CoV), severe acute respiratory syndrome CoV 2 (SARS-CoV-2), and swine enteric CoVs, including porcine epidemic diarrhoea virus (PEDs), transmissible gastroenteritis virus (TGEv), and a new bat-HKU2-like porcine CoVs.

No global network on coronaviruses has been formed yet, but one will soon be established (see the dedicated section under Chapter II). Once established, STAR-IDAZ IRC is planning to use the network as its Working Group (WG) on the topic, and to task the experts to identify research gaps and draft research roadmaps on the topic.

DISCONTOOLS

The development of the chapter on veterinary coronaviruses is in progress.

Recent developments

Recombinant chimeric transmissible gastroenteritis virus (TGEV)—porcine epidemic diarrhea virus (PEDV) virus provides protection against virulent PEDV (Pascual-Iglesias et al., 2019[27])

This article, published in Viruses, describes the engineering of a genetically defined live attenuated vaccine for porcine epidemic diarrhoea virus (PEDV). An attenuated virus was produced based on the transmissible gastroenteritis virus (TGEV) genome, expressing a chimeric spike protein from a virulent PEDV strain. This virus (rTGEV-RS-SPEDV) was attenuated in highly-sensitive five-day-old piglets, as infected animals did not lose weight and none of them died. In addition, the virus caused very minor tissue damage compared with a virulent virus. The rTGEV-RS-SPEDV vaccine candidate was also attenuated in three-week-old animals that were used to evaluate the protection conferred by this virus, compared with the protection induced by infection with a virulent PEDV US strain (PEDV-NVSL). The rTGEV-RS-SPEDV virus protected against challenge with a virulent PEDV strain, reducing challenge virus titres in jejunum and leading to undetectable challenge virus RNA levels in faeces. The rTGEV-RS-SPEDV virus induced a humoral immune response specific for PEDV, including neutralising antibodies. Altogether, the data indicated that rTGEV-RS-SPEDV could be a promising vaccine candidate against virulent PEDV infection.

Recombinant infectious bronchitis coronavirus H120 with the spike protein S1 gene of the nephropathogenic IBYZ strain remains attenuated but induces protective immunity (Jiang et al., 2020[28])

This article, published in Vaccine, describes the development of a recombinant infectious bronchitis (IB) vaccine. A recombinant strain, rH120-S1/YZ, was constructed using a reverse genetic system, based on the backbone of the H120 vaccine strain, with the spike protein S1 gene replaced with that of the QX-like nephropathogenic strain, ck/CH/IBYZ/2011, isolated in China. The results demonstrated that the biological

27 **Pascual-Iglesias, A., Sanchez, C. M., Penzes, Z., Sola, I., Enjuanes, L., & Zuñiga, S. (2019). Recombinant Chimeric Transmissible Gastroenteritis Virus (TGEV)—Porcine Epidemic Diarrhea Virus (PEDV) Virus Provides Protection against Virulent PEDV.** *Viruses*, **11(8), 682**.

28 **Jiang, Y., Cheng, X., Zhao, X., Yu, Y., Gao, M., & Zhou, S. (2020). Recombinant infectious bronchitis coronavirus H120 with the spike protein S1 gene of the nephropathogenic IBYZ strain remains attenuated but induces protective immunity. Vaccine, 38(15), 3157-3168.**

characteristics of the recombinant virus remained unchanged and no mortality, clinical signs, or lesions were observed in the lungs or kidneys of young chickens inoculated with rH120-S1/YZ, in contrast to the rIBYZ-infected group. The viral loads in various tissues, cloacal, and oral swabs was lower in most types of samples. The efficacy of rH120-S1/YZ was evaluated against the QX-like IBV strain; compared to rH120 vaccination group, it provided better protection, with 100% survival rate and no disease symptom or gross lesion in the chickens. Increased levels of IBV-specific antibodies were detected in the serum of the rH120-S1/YZ-vaccinated animals 14 days post-vaccination. The authors concluded that the recombinant strain, rH120-S1/YZ may represent a promising vaccine candidate against QX-like IBVs.

Infectious bronchitis virus evolution, diagnosis and control (Legnardi et al., 2020[29])

This article, published in Veterinary Sciences, reviews the main features of infectious bronchitis virus (IBV) biology and evolution, focusing on their relevance and potential applications in terms of diagnosis and control. The paper provides an overview of possible diagnostic approaches for IBV and guidance on the selection of the appropriate ones on a case-by case basis, and discusses relevant factors for IBV control.

Intramuscular immunization with chemokine-adjuvanted inactive porcine epidemic diarrhea virus induces substantial protection in pigs (Hsueh et al., 2020[30])

In this article, published in Vaccines, the authors investigated the use of porcine chemokine ligand proteins CCL25, 27, and 28 as novel adjuvants for porcine epidemic diarrhoea (PED) intramuscular vaccination. Different CC proteins were constructed and stably expressed in the mammalian expression system, and co-administered intramuscularly with inactivated PEDV (iPEDV) particles. The vaccination resulted in recruiting CCR9+ and/or CCR10+ inflammatory cells to the injection site, thereby inducing superior systemic PEDV specific IgG, faecal IgA, and viral neutralising antibodies in pigs. Moreover, pigs immunised with iPEDV in combination with CCL25 and CCL28 elicited substantial protection against a virulent PEDV challenge.

Porcine epidemic diarrhea virus (PEDV): An update on etiology, transmission, pathogenesis, and prevention and control (Jung et al., 2020[31])

This article, published in Virus research, reviews current knowledge on aetiology, transmission, pathogenesis, prevention, and control of porcine epidemic diarrhoea virus (PEDV) infection. Strategies for implementing effective biosecurity and immunoprophylaxis, both for infection prevention and control, are described in detail.

Susceptibility of turkeys, chickens and chicken embryos to SARS-CoV-2 (Berhane et al., 2020[32])

This article, published in Transboundary and emerging diseases, presents results from experimental infection studies on the susceptibility of turkeys, chickens and chicken embryos to SARS-CoV-2. Turkeys and chickens were inoculated using a combination of intranasal, oral and ocular routes, without developing clinical disease or seroconverting following inoculation. Viral RNA was not detected in oral swabs, cloacal swabs or in tissues using quantitative real-time RT-PCR. In addition, chicken embryos

29 **Legnardi, M., Tucciarone, C. M., Franzo, G., & Cecchinato, M. (2020). Infectious bronchitis virus evolution, diagnosis and control. Veterinary Sciences, 7(2), 79.**

30 **Hsueh, F. C., Chang, Y. C., Kao, C. F., Hsu, C. W., & Chang, H. W. (2020). Intramuscular immunization with chemokine-adjuvanted inactive porcine epidemic diarrhea virus induces substantial protection in pigs. Vaccines, 8(1), 102.**

31 **Jung, K., Saif, L. J., & Wang, Q. (2020). Porcine epidemic diarrhea virus (PEDV): An update on etiology, transmission, pathogenesis, and prevention and control. Virus research, 198045.**

32 **Berhane, Y., Suderman, M., Babiuk, S., & Pickering, B. (2020). Susceptibility of turkeys, chickens and chicken embryos to SARS-CoV-2. Transboundary and emerging diseases.(in press)**

were inoculated by various routes, failing to support replication of the virus in all instances. The authors concluded that SARS-CoV-2 does not affect turkeys or chickens in the current genetic state and does not pose any potential risk to establish an infection in both species of domestic poultry.

Susceptibility of swine cells and domestic pigs to SARS-CoV-2 (Meekins et al., 2020[33])

In this article, published in Emerging microbes & infections, the authors investigated the ability of SARS-CoV-2 to (i) replicate in porcine cell lines, (ii) establish infection in domestic pigs via experimental oral/intranasal/intratracheal inoculation, and (iii) transmit to co-housed naïve sentinel pigs. SARS-CoV-2 was able to replicate in two different porcine cell lines with cytopathic effects. Interestingly, none of the SARS-CoV-2-inoculated pigs showed evidence of clinical signs, viral replication, or SARS-CoV-2-specific antibody responses. Moreover, none of the sentinel pigs displayed markers of SARS-CoV-2 infection. These data indicate that, although different porcine cell lines are permissive to SARS-CoV-2, five-week old pigs are not susceptible to infection via oral/intranasal/intratracheal challenge. Thus, the authors concluded that pigs are therefore unlikely to be significant carriers of SARS-CoV-2 and are not a suitable pre-clinical animal model to study SARS-CoV-2 pathogenesis or efficacy of respective vaccines or therapeutics.

Transmission of SARS-CoV-2 on mink farms between humans and mink and back to humans (Munnink et al., 2020[34])

This article, published in Science, describes an in-depth investigation using whole-genome sequencing of outbreaks on 16 mink farms and the humans living or working on these farms. The authors concluded that the virus was initially introduced by humans and has since evolved, most likely reflecting widespread circulation among mink in the beginning of the infection period, several weeks before detection. Despite enhanced biosecurity, early warning surveillance, and immediate culling of animals in affected farms, transmission occurred between mink farms in three large transmission clusters with unknown modes of transmission. Of the tested mink farm residents, employees, and/or individuals with whom they had been in contact, 68% had evidence of SARS-CoV-2 infection. Individuals for which whole genomes were available were shown to have been infected with strains with an animal sequence signature, providing evidence of animal-to-human transmission of SARS-CoV-2 within mink farms.

Experimental infection of cattle with SARS-CoV-2 (Ulrich et al., 2020[35])

This article, published in Emerging infectious diseases, investigated the susceptibility of cattle to SARS-CoV-2 infection in experimental conditions. Six cattle were inoculated with cattle with SARS-CoV-2 and kept together with three uninoculated cattle. Low levels of viral replication and specific seroreactivity was observed in 2 inoculated animals, despite high levels of preexisting antibody titres against a bovine betacoronavirus. The in-contact animals did not become infected. Based on these results, the authors concluded that, under experimental conditions, cattle show low susceptibility to SARS-CoV-2 infection.

33 **Meekins, D. A., Morozov, I., Trujillo, J. D., Gaudreault, N. N., Bold, D., Carossino, M., ... & Richt, J. A. (2020). Susceptibility of swine cells and domestic pigs to SARS-CoV-2. Emerging microbes & infections, 9(1), 2278-2288.**

34 **Munnink, B. B. O., Sikkema, R. S., Nieuwenhuijse, D. F., Molenaar, R. J., Munger, E., Molenkamp, R., ... & Koopmans, M. P. (2020). Transmission of SARS-CoV-2 on mink farms between humans and mink and back to humans. Science, 371(6525), 172-177.**

35 **Ulrich, L., Wernike, K., Hoffmann, D., Mettenleiter, T. C., & Beer, M. (2020). Experimental infection of cattle with SARS-CoV-2. Emerging infectious diseases, 26(12), 2979.**

Susceptibility of domestic swine to experimental infection with Severe Acute Respiratory Syndrome Coronavirus 2 (Pickering et al., 2021[36])

This article, published in Emerging infectious diseases, investigated the susceptibility of pigs to SARS-CoV-2 infection. After oronasal inoculation of a high dose (1×10^6 PFU/animal) of SARS-CoV-2 to 16 pigs, the authors detected viral RNA in group oral fluids and in nasal wash from 2 pigs, while live virus was isolated from only 1 pig. Antibodies also were detected in only 2 animals at 11 and 13 days post-inoculation but were detected in oral fluid samples at 6 days post-inoculation, indicating antibody secretion. The authors concluded that, under these experimental conditions, pigs are susceptible to SARS-CoV-2 infection.

Ongoing research

Non-exhaustive list of ongoing projects on CoVs funded by STAR-IDAZ IRC and STAR-IDAZ Network Members:

- Argentina (INTA):
  - Domestic and wildlife surveillance project for SARS-CoV-2 infection.France (ANSES):
  - Bats as reservoir of orofecal pathogens with the main objective: epidemiological assessment of circulation of leptospirosis and virus (coronavirus and others) in bats.
  - Development and validation of animal models (ferrets and hamsters) of SARS-CoV2 infection to test therapeutics and preventive molecules.
  - In vivo assays in hACE2 transgenic mice of blocking peptides; Identification of antiviral molecules.
  - Study of the role of cellular cyclophilins in coronavirus replication and in host restriction with two main objectives: understanding the mechanism in domestic animals and exploring it in wildlife.
- France (INRAE):
  - Application of single-cell RNAseq to explore the diversity of cellular innate immune responses in the pig and avian lung.
  - Development/use of Fluidigm qPCR arrays to assess innate immune gene expression patterns in the pig and avian lung.
  - Envisaged use of Fluidigm qPCR arrays to assess innate immune gene expression patterns in lung tissue samples from SARS-CoV-2-infected pigs.
  - Use of porcine precision-cut lung slices (PCLS) to study host-pathogen interactions in the pig and avian lung.
  - Development of a set of immortalised swine bronchial epithelial cell lines to study virus-host interactions and the diversity of epithelial cell responses (funded by H2020 INFRAIA VetBioNet).
- Germany (FLI):
  - Implementing SARS-CoV-2 animal infection studies.
- Hungary (NAK):
  - Epidemiology and genetic changes of TGEV and PEDV coronavirus diseases.
  - Investigation of IBV genetics and development of pancoronavirus detection via NGS adaptation.

36 **Pickering, B. S., Smith, G., Pinette, M. M., Embury-Hyatt, C., Moffat, E., Marszal, P., & Lewis, C. E. (2021). Susceptibility of Domestic Swine to Experimental Infection with Severe Acute Respiratory Syndrome Coronavirus 2. Emerging infectious diseases, 27(1), 104.**

- Italy (MINSAL):
  - Susceptibility of mammals to SARS-CoV-2: risks of reverse zoonosis and possibilities in translational medicine.
- Netherlands (Royal GD):
  - IBV vaccine development projects (in collaboration with industry).
  - Pathogenesis testing of current and new IBV strains, *in-ovo*, *in-vitro* and *in-vivo*.
  - Testing of live and inactivated IBV vaccines in vaccination/challenge experiments, against different serotypes, interference between vaccines.
- Spain (INIA):
  - Characterise the new variants of PEDV in Spain and the immune response that they generate: investigate the hypothetical presence of other viruses associated to PEDV in outbreaks of diarrhoea and development of diagnostic and research techniques for the PEDV and other associated viruses.UK (BBSRC):
  - Analysis of the function of infectious bronchitis virus accessory proteins.
  - Determination of cross protection and genetic plasticity of IBV with the aim to control the virus.
  - Development of rationally attenuated live vaccines for effective control of infectious bronchitis.
  - Investigation on how passage in eggs results in attenuation, is this due to selection or random mutation with the aim to reduce the risk of vaccine reversion.
  - MERS-CoV as a model system as a classical three species zoonotic event: examining how MERS-CoV affects gene and protein expression in human, camel and bat cells and to study the interaction of key MERS-CoV proteins in these species.
  - Modification of the infectious bronchitis virus spike protein for growth in Vero cells; potential for vaccine growth and production in cell culture.
  - Molecular characterisation of the avian coronavirus infectious bronchitis virus to identify regions of the genome involved in virulence.
  - Strategically funded institute research into Porcine Deltacoronavirus (PDCoV): characterising virus induced replication organelles, understanding the process of viral envelopment and identifying cellular proteins that are important for virus replication.
- UK (Defra):
  - Investigation of SARS-CoV-2 survival on surfaces and transmission risk (e.g., animal fur).
  - Molecular and sero-based test development for SARS-CoV-2.

8. Vector-borne diseases (VBD)

Global network

No global network on vector-borne diseases has been formed yet. Due to the width of the topic, which involves several diseases affecting different animal species and humans (*e.g.* Rift Valley fever), it was decided to focus the activities of the STAR-IDAZ IRC Working Group (WG) on vectors (*i.e.* insects and ticks) including pathogen transmission by the vector, rather than on specific diseases. Working Group members are being identified through nominations by the STAR-IDAZ IRC Executive and Scientific Committees. In the meantime, SIRCAH collaborated with the STAR-IDAZ IRC Scientific Committee to draft a research roadmap for “Vector transmission and control”

In order to showcase recent developments on VBD research, foster closer collaboration, and to introduce the roadmap for vector transmission and control, STAR-IDAZ IRC participated in the UK Vector Borne Disease Conference, organised by the University of Liverpool, UK. The event was held as a series of webinars in November 2020, and STAR-IDAZ IRC co-organised one of them (on the 23rd of November). There, along with a few invited scientific presentations and a general introduction to the STAR-IDAZ IRC, one talk was dedicated to introducing and discussing the research roadmap for focusing the research effort on vector transmission and control. When the list of members of the WG is finalised, a virtual workshop will be convened to validate the roadmaps, and possibly form subgroups to focus on the main topics that are defined in this first meeting. The first meeting is expected to take place in 2021.

DISCONTOOLS

The database contains information about several VBD (*i.e.,* African horse sickness, African trypanosomoses, bluetongue, Crimean-Congo haemorrhagic fever, Rift Valley fever, theileriosis, West Nile virus). The disease specific information is available at **www.discontools.eu** and the summaries are available in the **DISCONTOOLS e-book.**

Recent developments

Even if the scope of the STAR-IDAZ IRC WG on VBD has been defined, the topic is too broad to be adequately covered here. This session of the report will be further developed after the establishment of the WG, in order to obtain support in the identification of relevant publications.

Ongoing research

Even if the scope of the STAR-IDAZ IRC WG on VBD has been defined, the topic is too broad to be adequately covered here. This session of the report will be further developed after the establishment of the WG, in order to obtain support in the identification of relevant projects.

8. Antimicrobial resistance – Alternative to antibiotics

Global network

Antimicrobial resistance was identified as one of the priority issues by the STAR-IDAZ IRC, and it was decided that a Working Group (WG) would be established to identify research gaps on this issue. Nevertheless, the topic is extremely broad and multifaceted, and many other initiatives are already existing in this area. In order to ensure progress and avoid duplication, it was decided that the STAR-IDAZ IRC WG should focus on the development of alternatives to antibiotics, where R&D was still a major need, and global coordination was lacking.

The STAR-IDAZ IRC WG was established, in 2019 to identify research needs to support the development of alternatives to antibiotics and the reduction/rationalisation of the use of antimicrobials in livestock. This will ultimately help in the development of new non antibiotic-based antimicrobial products and approaches for controlling infections and enhancing productivity, while maximising the life of existing and new therapeutics.

A first physical meeting of the WG was held in Bangkok, Thailand in December 2019, back-to-back with the 3rd International Symposium on Alternatives to Antibiotics (ATA). Participants completed a questionnaire beforehand, which formed the basis of discussion at the workshop. The questionnaire covered the research needs on four main areas, i. alternatives to antibiotics acting directly on the pathogen, including establishing their mode of action (focusing on phages), ii. agents/compounds for their ability to enhance the hosts resistance to disease, including establishing their mode of action (focusing on immunomodulators),

iii. mode of action of antibiotics as growth promoters and, iv. role of microbiome in the maintenance of health and how it might be manipulated. For each topic, experts were asked to identify the main research questions, research needs, possible solution routes, and dependencies.

Following the first workshop, the WG was divided into 4 subgroups focussing on: i) phages, ii) immunomodulators, iii) mode of action of antibiotics as growth promoters and iv) the microbiota. Some experts are involved in multiple subgroups. A gap analysis and research roadmap workshop was planned alongside the annual meeting of the European Federation of Animal Science (EAAP) in Porto, Portugal in September 2020. However, when this was postponed and rescheduled as a virtual meeting, the STAR-IDAZ IRC decided to advance the work of the WG independently. Thus, three webinars were held in early October 2020, to progress the gap analysis and develop the draft roadmaps on microbiota, immunomodulators, and phages. Inputs from these meetings are currently being analysed to finalise the work on gap analysis; the next steps will be to hold a webinar to draft the roadmap for growth promoters and to organise a final workshop, hopefully face-to-face, to finalise the four roadmaps.

DISCONTOOLS

DISCONTOOLS does not cover antimicrobial resistance or alternatives to antibiotics as a separate topic, but disease specific information on effectiveness of control tools can be retrieved via targeted search in the **database**.

Recent developments

Sex pilus specific bacteriophage to drive bacterial population towards antibiotic sensitivity (Colom et al., 2019[37])

Is this paper, published in Scientific Reports, the authors describe their study on the use of sex pilus-specific (SPS) phage to reduce the carriage of antimicrobial resistance (AMR) plasmids. In some AMR bacteria, resistance is encoded by conjugative plasmids expressing sex-pili that can readily spread resistance through bacterial populations. The authors found that SPS phage can kill AMR *Escherichia coli* and select for AMR plasmid loss *in vitro*. In addition, they demonstrated that SPS phage can both prevent the spread of AMR *Salmonella* Enteritidis infection in chickens and shift the bacterial population towards antibiotic sensitivity.

Strategic priorities for research on antibiotic alternatives in animal agriculture - results from an expert workshop (Kurt et al., 2019[38])

This paper, published in Frontiers in veterinary science, presents the results from an expert workshop that aimed at identifying strategic priorities for funding research and development on alternatives to antibiotics (ATA) for livestock. The workshop explored factors critical to the success or failure of new ATA and identified associated data gaps and research needs that, if addressed, could help grow the pipeline of safe and effective product candidates. In addition, the workshop allowed developing a framework to enable a consistent, systematic, and transparent evaluation of ATA candidates. This evaluation framework takes into account both the economic viability (including development costs and expected revenues) and the evaluation of potential risks (including on safety, efficacy, acceptability, and practicability) related to the new products, and is described in detail in the paper.

37 **Colom, J., Batista, D., Baig, A., Tang, Y., Liu, S., Yuan, F., ... & Barrow, P. (2019). Sex pilus specific bacteriophage to drive bacterial population towards antibiotic sensitivity. Scientific reports, 9(1), 1-11.**

38 **Kurt, T., Wong, N., Fowler, H., Gay, C., Lillehoj, H., Plummer, P., ... & Hoelzer, K. (2019). Strategic priorities for research on antibiotic alternatives in animal agriculture—Results from an expert workshop. Frontiers in veterinary science, 6, 429.**

Potential dietary feed additives with antibacterial effects and their impact on performance of weaned piglets: A meta-analysis (Vanrolleghem et al., 2019[39])

A review, published in The Veterinary Journal, presents a meta-analysis evaluating the use of potential dietary feed additives (pDFA) with antibacterial effects and their impact on the performance of weaned piglets. The pDFA in the analysed studies (n=23) could be grouped in 5 classes: antimicrobial peptides, chitosan, lysozyme, medium chain fatty acids/ triglycerides, and plant extracts. For each class of pDFA, results of the meta-analysis showed significantly higher average daily gain in the group with pDFA compared to the negative control group, while no significant difference with the positive control group was observed. Furthermore, a positive effect on food conversion rate was found in the group with pDFA compared to the negative control group. Overall, these results suggest that pDFA could reduce the use of antimicrobials without significant negative effects on performance indicators.

Probiotics in animal husbandry: applicability and associated risk factors (Alayande et al., 2020[40])

A review, published in the journal Sustainability, investigated the effectiveness of probiotics as a safe and viable alternative to antibiotics for increasing performance in livestock. Besides improved immunomodulation and nutrient digestibility, in-feed probiotics have shown drastic reductions in gastrointestinal tract-invading pathogens. The paper also highlights a few risk factors that need to be considered when developing probiotics (e.g., presence of virulence determinants, possibility of promoting deleterious metabolic effects, excessive immune stimulation, genetic stability of the strains), in order to ensure food safety and security.

Viruses of protozoan parasites and viral therapy: Is the time now right? (Barrow et al., 2020[41])

This review, published in Virology Journal, presents and discusses the potential for exploiting viruses of medical importance identified in protozoan parasites to treat parasitic diseases together with the challenges associated with their application. The paper presents rationally driven strategies to employ these viruses successfully against parasitic diseases and discussed them in the light of the current knowledge of the virus biology and the complex interplay between the viruses, the parasite hosts and the human/animal host. Lastly, the authors highlight knowledge gaps that should be addressed to advance the potential of virotherapy against parasitic diseases.

Efficacy of experimental phage therapies in livestock (Dec et al., 2020[42])

This review, published in Animal Health Research Reviews, investigates the history of phage therapy as a replacement for antibiotics in livestock, presenting current examples and results of experimental phage treatments in comparison to antibiotics.

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39 **Vanrolleghem, W., Tanghe, S., Verstringe, S., Bruggeman, G., Papadopoulos, D., Trevisi, P., ... & Dewulf, J. (2019). Potential dietary feed additives with antibacterial effects and their impact on performance of weaned piglets: a meta-analysis. The Veterinary Journal, 249, 24-32.**

40 **Alayande, K. A., Aiyegoro, O. A., & Ateba, C. N. (2020). Probiotics in animal husbandry: Applicability and associated risk factors. Sustainability, 12(3), 1087.**

41 **Barrow, P., Dujardin, J. C., Fasel, N., Greenwood, A. D., Osterrieder, K., Lomonossoff, G., ... & Lalle, M. (2020). Viruses of protozoan parasites and viral therapy: Is the time now right?.** *Virology Journal*, *17*(1), 1-14.

42 **Dec, M., Wernicki, A., & Urban-Chmiel, R. (2020). Efficacy of experimental phage therapies in livestock. Animal Health Research Reviews, 21(1), 69-83.**

Last call for replacement of antimicrobials in animal production: modern challenges, opportunities, and potential solutions (Nowakiewicz et al., 2020[43])

This paper, published in Antibiotics, provides an overview of the antimicrobial resistance phenomenon and its determinants, the steps taken to solve the problem, including the introduction of alternatives to antibiotics, and the evaluation of some factors influencing the potential implementation of such alternatives in animal production. The review also presents two groups of alternatives (bacteriophages and antimicrobial peptides), which, given their mechanism of action and spectrum, are, in the view of the authors, most comparable to the effectiveness of antibiotics.

Ongoing research

Non-exhaustive list of ongoing projects on PRRS funded by STAR-IDAZ IRC and STAR-IDAZ Network Members:

- European Commission:
  - AVANT (**https://avant-project.eu/**): development of alternatives to antimicrobials for the management of bacterial infections in pigs, especially diarrhoea during the weaning period, as the major indication for antimicrobial use in livestock in Europe.
  - HealthyLivestock (**https://healthylivestock.net/**): a research programme to study the contributions of enhanced animal health and welfare on reducing the need to use antimicrobials in pigs and poultry.
  - PHAGOVET (**https://www.phagovet.eu/**): investigating phages as a cost-effective solution for controlling Salmonella and *Escherichia coli* in poultry production.
  - APT (**https://armentavet.com/about-us/**): to address the issue of bovine mastitis, developed an innovative, non-invasive and antibiotics-free therapy that has proven to increase milk yield and quality, as well as reduce culling, using acoustic pulse technology (APT).

43 **Nowakiewicz, A., Zięba, P., Gnat, S., & Matuszewski, Ł. (2020). Last Call for Replacement of Antimicrobials in Animal Production: Modern Challenges, Opportunities, and Potential Solutions. Antibiotics, 9(12), 883**.