STAR-IDAZ
International Research
Consortium on Animal Health

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

DELIVERABLE 4.4:
October 2019

The Secretariat for the STAR-IDAZ IRC (SIRCAH) is funded from the European Union's Horizon 2020 research and innovation programme under grant agreement No 727494

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This document was produced by the Scientific Secretariat of the STAR-IDAZ International Research Consortium on Animal Health (SIRCAH). SIRCAH is supported, since 2016, by a European H2020 contract, "Secretariat for the International Research Consortium on Animal Health", which schedules the annual publication of a report on the state-of-the art on animal health research on IRC priorities. The report aims to inform stakeholders at large of developments in the field of IRC priority diseases and support decisions of policy makers and research funders.

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 reflect the current position.

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 member.

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 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. Lastly, a selection of promising innovations or major research outcomes, published in scientific journals over the past three years were identified through a scan of the scientific literature in the CAB Abstracts database using specific key words for each of the priority diseases.

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 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 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:

- Establish a Global Coordination Mechanism to facilitate a regular dialogue among main stakeholders for both R&D preparedness and response;
- Prepare a MOU with Global Research Collaboration for Infectious Disease Preparedness (GloPID) to facilitate collaboration with funders of research on emerging diseases;
- Facilitate the compilation and maintenance of an interactive list of key stakeholders and a database of research preparedness resources;
- 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.

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 World Health Organization, 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 has now secured $750 million toward its $1billion funding target, with multi-year funding from Norway, Germany, Japan, Canada, Australia, the Bill & Melinda Gates Foundation, and Wellcome. CEPI has also received single-year investments from the governments of Belgium and the UK. The European Commission foresees substantial financial contributions to support relevant projects through its mechanisms. CEPI Secretariat is based in Oslo, Norway.

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.

At first, CEPI will focus on vaccines for known epidemic threats; the diseases will be selected by CEPI's scientific advisory committee based on the priority list of pathogens outlined in the WHO R&D Blueprint for Action to Prevent Epidemics, which is updated yearly.

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.

CEPI is primarily focussing on 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).

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 will be 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 global level.

Global Alliance for Livestock Veterinary Medicines (GALVmed)

https://www.galvmed.org/

The Global Alliance for Livestock Veterinary Medicines (GALVmed) is a not-for-profit livestock health product development & adoption organisation working with and through partners to make livestock vaccines, medicines and diagnostics accessible to the millions for whom livestock is a lifeline. It concentrates on livestock diseases of major economic importance to small-scale livestock producers, including such diseases as East Coast fever (ECF), Newcastle disease (ND), contagious bovine pleuropneumonia (CBPP) and peste des petits ruminants (PPR).

GALVmed was formally established in 2005, with initial funding from the UK Government Department for International Development (DfID). By 2008, funding from Bill & Melinda Gates Foundation 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, and delivered 8 proofs-of concept and 10 products.

The Members of GALVmed are from a wide range of public bodies, private institutions including pharmaceutical companies and non-governmental organisations. Observers from key donors and partners, including the World Organisation for Animal Health (OIE), are invited to attend GALVmed board meetings.

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.

The main projects and programmes have targeted African swine fever (ASF), African trypanosomoses, brucellosis, CBPP, contagious caprine pleuropneumonia (CPPP), ECF, lumpy skin disease (LSD), ND, PPR, porcine cysticercosis, and Rift Valley fever (RVF).

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.

'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.

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 one-half of this budget is provided 'in kind' by the EPFIA (pharmaceutical industry association) companies that are participating in the projects.

IMI today has over 50 projects, with more in the pipeline. While the main emphasis of these projects is on human health, some focus is on broad challenges in drug development, such as drug and vaccine safety, the sustainability of chemical drug production, the use of stem cells for drug discovery, and antimicrobial resistance. 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.

Zoonoses Anticipation and Preparedness Initiative (ZAPI)

http://zapi-imi.eu/

The Zoonoses Anticipation and Preparedness Initiative (ZAPI) is part 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 euros, 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.

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 itslef. 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.

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 poor livestock 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 the Bill & Melinda Gates Foundation, Global Affairs Canada and Canada's International Development Research Centre. 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).

Interagency Coordination Group on Antimicrobial Resistance (IACG)

http://www.who.int/antimicrobial-resistance/interagency-coordination-group/en/

The Interagency Coordination Group on Antimicrobial Resistance (IACG) is an ad hoc group of high-level experts established by the UN Secretary-General in 2017 to provide practical guidance on approaches needed to ensure sustained effective global action to address antimicrobial resistance (AMR).

At its first meeting, that was held at the Headquarters of the World Organisation for Animal Health (OIE) in October 2017, the IACG established six Subgroups as a way to advance analysis of specific issues and develop preliminary recommendations to feed into IACG's plenary sessions.

Subgroup 4 covers innovation and research and boosting R&D and access. The final aim of this Subgroup is to work towards high-level recommendations (e.g. targeting the UN Secretary-General) on how to operationalise the global response to AMR. The recommendations focus on prioritisation to guide investments on, and propose principles and strategies to stimulate, R&D and access.

The subgroup delivered a first discussion paper, that was opened to public consultation, in June 2018. The aim of the paper was to identify challenges and gaps facing R&D and access to AMR-related health technologies and to invite discussion on how to address these in the framework of a global response. Based on the outcomes, the subgroup developed practical recommendations to address challenges to AMR R&D and access, which were delivered to the IACG Secretariat.

On 29 April 2019, after discussion with all of the subgroup, and harmonisation of the document, the IACG Secretariat submitted the Recommendations Report to the Secretary-General of the UN. The report contains critical recommendations to combat drug-resistant infections and that demand immediate, coordinated and ambitious action to avert a potentially disastrous drug-resistance crisis. The final report is available at https://www.who.int/antimicrobial-resistance/interagency-coordination-group/IACG_final_report_EN.pdf?ua=1.

The IACG has now completed its mandate.

Foot and Mouth Disease (FMD) Challenge Project

(website not yet available)

AgResults is about to start a new competition for encouraging the development and uptake of an improved vaccine specifically for the needs of East Africa, the Foot and Mouth Disease (FMD) Challenge Project, that will be managed by GALVmed.

The FMD 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 kick off meeting of the project will be held alongside the EuFMD Vaccine Security Meeting, on the 22 January 2020.

II. INTERNATIONAL INITIATIVES TO FACILITATE TRANSNATIONAL 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 address 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 ICRAD ERA-NET (International Coordination of Research on Infectious Animal Diseases, 2019 - 2023).

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

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. The network now moves 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.

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.

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 (a first call has been launched already, and 11 projects were selected). 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).

Global research networks on specific diseases

(websites for the specific networks, where 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. Further details on the specific networks for the 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).

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. Funding for these activities (£2.1M) was provided in 2017 by the UK Medical Research Council and the Biotechnology and Biological Sciences Research Council.

The objectives of the International Veterinary Vaccinology Network 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 LMIC-relevant pathogens;
- Advance the development of veterinary vaccines for LMIC-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 LMIC countries, to ensure the sustainable delivery of effective vaccines.

Built on the basis of the UK Veterinary Vaccinology Network, the IVVN has to date more than 1,000 members.

African Vaccinology Network (AfVANET)

(website not yet available)

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.

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.

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 started out life as an EU FP7-funded project in 2008. Today, 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. As such, the website received a facelift in 2018 and the database has become an important resource for funders of animal health research and the research community in general to develop research agendas, and evaluate research proposals. The DISCONTOOLS research gap analyses are used as a ground layer for the building of STAR-IDAZ road maps of research.

Veterinary Biocontained research facility Network (VetBio-Net)

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 28 academic and industrial partners from 12 countries across Europe, Africa, and Oceania. VetBioNet's principal objectives are to reinforce the cooperation between Europe's leading high-containment research infrastructures, to provide access to the high-end research facilities of the network, and to further improve the technical standard of the services provided. VetBioNet will serve as a multidisciplinary network seeking to drive the European Research & Development agenda related to emerging epizootic and zoonotic diseases. 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.

Global Open Data for Agriculture and Nutrition (GODAN)

http://www.godan.info/

The Global Open Data for Agriculture and Nutrition (GODAN) initiative is a voluntary association having the purpose of supporting the proactive sharing of open data to make information about agriculture and nutrition available, accessible and usable to deal with the urgent challenge of ensuring world food security. GODAN was launched in 2013 and now counts over 579 partners from national governments, non-governmental, international and private sector organisations that have committed to a joint Statement of Purpose. STAR-IDAZ is a partner of the initiative.

The sharing and using of data would allow saving resources and enhancing research efficiency, accelerating the delivery of results. The GODAN initiative focuses on building high-level support among governments, policymakers, international organisations and business, and promotes collaboration to manage the growing volume of data generated by new technologies, so as to solve long-standing problems and to benefit farmers and the health of consumers.

With a focus on open data for agriculture and nutrition, GODAN seeks to:

- Advocate for new and existing open data initiatives to set a core focus on agriculture and nutrition data;
- Encourage the agreement on and release of a common set of agricultural and nutrition data;
- Increase widespread awareness of ongoing activities, innovations and good practices;
- Advocate for collaborative efforts on future agriculture and nutrition open data endeavours; and
- Advocate programmes, good practices, and lessons learned that enable the use of open data particularly by and for the rural and urban poor.

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).

The EVAg consortium includes an international group of 26 laboratories, 19 belonging to EU Member States' institutions and 7 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.

The EVAg global virus collection is a valuable support tool for the organisation for 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.

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

https://www.gesundheitsforschung-bmbf.de/en/GlobalAMRHub.php

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 (12 September 2017). 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 will be a close to real-time Dynamic Dashboard providing information and analysis at a high level on current initiatives, funding flows and activities in the field of AMR R&D. The information will be analysed and shall inform policy makers in their decision making on strategic investments and actions in AMR R&D.

This information will be publicly available. A prerequisite for achieving this goal is to have a full picture of the AMR R&D landscape, including all available mappings shared by other contributing organisations (such as WHO) actively working to combat AMR.

The Global AMR R&D Hub is currently focussing its efforts on bacterial infections for humans; animal health will start being considered in 2020.

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 were identified for which research coordination is required to make progress and deliver the control tools that are needed. This preliminary list was further discussed during the meeting of the STAR-IDAZ IRC Executive and Scientific Committees' meetings that were held in Nairobi on the 30-31 January 2017. The full list of the currently identified priorities is reported below.

- African Swine Fever (ASF)
- Animal genomics/genetics for animal health
- Bovine Tuberculosis (bTB)
- Brucellosis
- Coronaviruses
- Diagnostics (tools and technologies)
- Emerging issues
- Epidemiology
- Foot and Mouth Disease (FMD)
- Foresight
- Helminths
- Vaccinology
- Influenza
- AMR and the Development of Innovative Alternatives
- Mastitis
- One Health
- Porcine Reproductive and Respiratory Syndrome (PRRS)
- Porcine Respiratory Disease Complex (PRDC)
- Pox virus infections
- Vector-borne diseases

In the framework of that same meeting, 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 that was held in Madrid on the 14-15th of March 2018, it was agreed that coronaviruses and vector-borne diseases be added to the list of those being taken forward. At the 3rd STAR-IDAZ IRC Executive Committee meeting that was held in Beijing on the 13-14th of March 2019, the scope of the work to be performed on vector-borne diseases was better defined, and it was decided to also start looking into antimicrobial resistance (AMR) and the development of innovative alternatives to antibiotics.

Other priorities discussed during the above-mentioned Executive Committee meetings, included vaccinology, innovative anti-infective approaches including alternatives to antibiotics, and diagnostics. These will not be covered in this session of the report, due to their horizontal reach.

In the 2017 edition of the State-of-the-art report, this section focussed on the topics selected to be addressed first. In the yearly updates of the report, information on the diseases already being addressed is updated and sections are included for the newly selected diseases.

For each of the selected diseases, this report will provide an overview of the research situation at the global level, providing information on:

1. Existing or planned global networks that aim at guiding future research on the topic, and that are acting as STAR-IDAZ IRC Working Groups (see below);
2. Identified gaps on control tools (diagnostics, vaccines and pharmaceuticals), extracted from the DISCONTOOLS database;
3. Recent research advances, focussing on the past 3 years (i.e. for the first report, the period 2015-2017 was covered), providing an overview of a selection of highly relevant papers on the subject matter.

Concerning the existing global research networks, it is important to point out that STAR-IDAZ IRC is now establishing its Working Groups (WGs), which are being tasked to identify research gaps and draw research roadmaps on the priority diseases. When pre-existing networks are not available the section of this chapter on 'global network' will describe the STAR-IDAZ IRC WG on the specific disease. The rules for selecting the experts that form STAR-IDAZ IRC WGs are described in the WG ToR.

For what concerns the selection of articles to be outlined in the recent research advances section, we reviewed the literature published on the priority diseases that have been identified and have 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.

Further reports will also provide information on ongoing research on the topic conducted by the IRC Members, extracted by the STAR-IDAZ IRC project database, when this will be fully operational for all member countries. This section has not been implemented in the current version of the report.

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, 33 partners coming from all regions of the world and several stakeholders, including STAR-IDAZ.

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;
- 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.

The last meeting was held in Cagliari (Italy) in April 2018. The meeting was organised in collaboration with GARA, SIRCAH and the local hosting institution (Istituto Zooprofilattico Sperimentale della Sardegna). One of the aims of the meeting was to update the gap analysis that was conducted during the previous GARA workshop, which was held in Ploufragan (France) in 2016; the revised document is available on the GARA website[1]. In addition, during the Cagliari meeting the experts discussed, under the guidance of SIRCAH, the draft of an ASF vaccine research roadmap, initially prepared using information from the gap analyses of the Ploufragan workshop. The roadmap, now revised based on the inputs received by the experts, has been published on the STAR-IDAZ IRC website, together with a roadmap for the development of ASF diagnostic tests. GARA experts have now been contacted to provide a final validation of the roadmaps, and to prioritise the emerged research needs.

The next GARA meeting is planned to be held in Kampala, Uganda, on 14-16th April 2020.

DISCONTOOLS

The information on ASF in the DISCONTOOLS database was updated in April 2015 and some of the identified gaps in the field of diagnostics, vaccines and pharmaceuticals have been included below. Other knowledge gaps and more information are available at www.discontools.eu.

Diagnostics

Currently a number of good and fast diagnostic tools are available for both virus and antibody detection. Most of the existing tools allow early detection of the disease and a confident diagnosis in any epidemiological situation of African and European affected countries. An increasing number of commercial kits (serology, PCR) have become available in the last few years. The new validated real time PCRs have been shown to provide higher sensitivity for the detection of carrier animals surviving the infection. On-site first-line tools have been developed and there are validated commercial tests available. Nevertheless, ASF diagnosis is complex and some gaps and needs remain. Some epidemiological information and virus transmission characteristics are gaps of great importance because they influence the strategy, quality and reliability of ASF diagnosis. These needs include: i) expansion of field validation for all tests and appropriate specimens; ii) standardisation and validation of ASF diagnosis in alternative types of samples; iii) established cell lines that make virus isolation a cost-effective test for its implementation at the National Reference Laboratories; iv) development of new diagnostic tools to assure the detection of survivor animals and carriers; and v) improvements in molecular characterisation tests to determine the source of the outbreaks.

To support surveillance and control/eradication programmes, the diagnosis of ASF should involve the simultaneous detection of specific antibodies and identification of the virus (DNA/Antigens) in the same animal.

Vaccines

Attempts over many years to develop inactivated or attenuated vaccines to ASF have failed. Conventional strategies for a vaccine have not been useful to date. Inactivated vaccines have conferred no protection. Attempts to attenuate the virus through passage in cell culture and/or macrophages induced some protection but are not totally safe. To date, DNA vaccine strategies have not been successful nor have the deletion mutant strategies. A better understanding of the immune response to infection and the humoral and cellular basis for the lifelong immunity post infection is needed with the identification of target proteins or genes.

Pharmaceuticals

There may be some potential for the use of antivirals in ASF control but there would be considerable problems in both developing and licensing such products.

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1 https://www.ars.usda.gov/ARSUserFiles/np103/SymposiumWorkshopsMeetings/GARA%20Gap%20Analysis%20Report%202018%2011-11-18.pdf

Recent developments

Deletion of African swine fever virus interferon inhibitors from the genome of a virulent isolate reduces virulence in domestic pigs and induces a protective response (Reis et al., 2016[2])

An article published in Vaccine investigates if the deletion of genes implied in the modulation of the type I interferon (IFN) response (i.e. genes from MGF360 and MGF530/505 families) in the genome of the virulent ASF virus isolate Benin 97/1 would affect virus attenuation and induction of protective immunity. The in vitro replication of the deletion mutant (BeninΔMGF) was similar to that of the parental virus and of the natural attenuated isolate OURT88/3, which has a similar deletion of genes. Levels of IFN-β in infected macrophages were higher for the deleted viruses as compared to the parental virus, confirming the role of MGF360 and MGF530/505 genes in suppressing IFN. The immunisation and boost of pigs with BeninΔMGF showed that the virus was attenuated, and all pigs were protected against challenge with a lethal dose of virulent Benin 97/1. The authors conclude that the deletion of IFN modulators would be a promising route for the construction of rationally attenuated ASFV candidate vaccine strains.

Development of a novel lateral flow assay for detection of African swine fever in blood (Sastre et al., 2016[3])

An article published in BMC veterinary research describes the development of a novel lateral flow assay (LFA) for detecting ASF antigens in blood. The test is based on the use of a monoclonal antibodies against ASF virus VP72 protein, the major viral capsid protein and highly immunogenic. Comparative tests were performed with both PCR and antigen-ELISA assay. The LFA sensitivity appeared to be well correlated with the ELISA one, but lower than the PCR one both on blood samples from experimentally infected pigs and field animals. For both groups of sera, LFA specificity was close to 100%. The authors conclude that this novel LFA test would allow rapid and reliable detection of ASF virus, representing a useful tool for control programmes and in situations where laboratory support and skilled personnel are limited.

Development of a new PCR assay for detecting ASF virus (Luo et al., 2017[4])

An article published in the Archives of Virology describes the development of a novel PCR assay for detection of ASF virus. Due to the lack of appropriate vaccines, the rapid and reliable detection of the virus is essential for timely implementation of emergency control measures, as well as to allow comparative diagnosis with other swine diseases. The authors designed primers specific for ASF virus based on the highly conserved region of the vp72 gene sequences and established a new PCR assay, which was then compared with two OIE-validated PCR tests. The novel test was applied on 14 strains of ASFV representing four genotypes (I, V, VIII and IX) from diverse geographical areas and on 62 clinical swine blood samples collected from Uganda, with good success. According to the authors, the novel PCR assay is specific, sensitive, and applicable for molecular diagnosis and surveillance of ASF.

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2 Reis, A. L., Abrams, C. C., Goatley, L. C., Netherton, C., Chapman, D. G., Sanchez-Cordon, P., & Dixon, L. K. (2016). Deletion of African swine fever virus interferon inhibitors from the genome of a virulent isolate reduces virulence in domestic pigs and induces a protective response. *Vaccine*, 34(39), 4698-4705.

3 Sastre, P., Gallardo, C., Monedero, A., Ruiz, T., Arias, M., Sanz, A., & Rueda, P. (2016). Development of a novel lateral flow assay for detection of African swine fever in blood. *BMC Veterinary Research*, 12(1), 206.

4 Luo, Y., Atim, S. A., Shao, L., Ayebazibwe, C., Sun, Y., Liu, Y., Ji, S., Meng, XY., Li, S., Li, Y., Masembe, C., Ståhl, K., Widén, F., Liu, L., Qiu, HJ. (2017). Development of an updated PCR assay for detection of African swine fever virus. *Archives of Virology*, 162(1), 191-199.

What challenges for ASF vaccine development? (Rock, 2017[5])

An article published in Veterinary Microbiology describes the challenges surrounding ASF vaccine design and development, with an emphasis on existing knowledge gaps. Since protection against reinfection with the homologous strain of ASF virus (ASFV) has been clearly demonstrated, vaccination is possible. Nevertheless, vaccine development is impeded by the large gaps of knowledge concerning ASFV infection and immunity, the extent of ASFV strain variation in nature and the identification of protective antigens. The review identifies the significant challenges remaining before delivering effective vaccines. The main challenge remains the identification of ASFV protein(s) responsible for inducing solid protective immune responses in the pig. To maximise live-attenuated vaccine safety without compromising immunogenicity, it will be necessary to identify a specific complement of attenuating mutations functioning in diverse ASFV genetic backgrounds. Relevant ASFV protective antigens and viral strain diversity in nature need to be known before designing ASF subunit or DIVA-compatible vectored vaccine strategies and evaluating delivery systems.

Characterization of the interaction of African swine fever virus with monocytes and derived macrophage subsets (Franzoni et al., 2017[6])

An article published in Veterinary Microbiology investigated, in vitro, the interaction of monocytes, un-activated (moMΦ), classically (moM1) and alternatively (moM2) activated monocyte-derived macrophages with a virulent (22653/14) and a non-pathogenic (BA71V) ASF virus (ASFV) strain. The ASFV primarily infects cells of the myeloid lineage and this tropism is thought to be crucial for disease pathogenesis. Using a multiplicity-of-infection (MOI) of 1, both viruses were able to infect monocytes and macrophage subsets, but BA71V presented a reduced ability to infect moM1 compared to 22653/14, with higher expression of early compared to late proteins. At lower MOI (0.01), only 22653/14 was able to replicate in all the macrophage subsets. No differences were observed in the expression of CD163 between ASFV infected and uninfected bystander cells. ASFV down-regulated CD16 expression but did not modulate MHC class II levels in monocytes and macrophage subsets. BA71V-infected but not 22653/14-infected moMΦ and moM2 showed a reduced expression of MHC class I compared to the mock-infected controls. Higher levels of IL-18, IL1-β and IL-1α were released from moM1 after infection with BA71V compared to 22653/14 or mock-infected control. The researchers concluded that virulent isolates have evolved mechanisms to counteract activated macrophage responses, promoting their survival, dissemination in the host and so ASF pathogenesis.

Adenovirus-vectored novel African Swine Fever Virus antigens elicit robust immune responses in swine (Lokhandwala et al., 2017[7])

In this article, published in PLoS One, the authors evaluated the immunogenicity of seven adenovirus-vectored novel ASFV antigens (i.e. A151R, B119 L, B602 L, EP402RΔPRR, B438 L, K205R, and A104R), as to identify candidates for rationally designing a prototype multi-antigen ASFV subunit vaccine. The study was conducted on commercial swine, that received a cocktail of the recombinant adenoviruses formulated in adjuvant and that underwent rapid recall upon boost. Most vaccinated animals mounted robust IgG responses against all the antigens in the cocktail. The induced antibodies recognised viral proteins from Georgia 2007/1 ASFV-infected cells by IFA and by western blot analysis. The recombinant adenovirus cocktail also induced ASFV-specific IFN-γ-secreting cells that were recalled upon boosting. Evaluation of local and systemic effects of the recombinant adenovirus cocktail post-priming and post-boosting in the immunised animals showed that the immunogen was well tolerated, and no serious negative effects were observed. The authors concluded that the adenovirus-vectored novel ASFV

5 Rock, D. L. (2017). Challenges for African swine fever vaccine development—"… perhaps the end of the beginning". *Veterinary Microbiology*, 206, 52-58.

6 Franzoni, G., Graham, S. P., Dei Giudici, S., Bonelli, P., Pilo, G., Anfossi, A. G., ... & Oggiano, A. (2017). Characterization of the interaction of African swine fever virus with monocytes and derived macrophage subsets. *Veterinary Microbiology*, 198, 88-98.

7 Lokhandwala, S., Waghela, S. D., Bray, J., Sangewar, N., Charendoff, C., Martin, C. L., ... & Brake, D. (2017). Adenovirus-vectored novel African Swine Fever Virus antigens elicit robust immune responses in swine. *PloS One*, 12(5), e0177007.

antigen cocktail was capable of safely inducing strong antibody and IFN-γ+ cell responses in commercial swine. They proposed data be used for selection of antigens for inclusion in a multi-antigen prototype vaccine to be evaluated for protective efficacy.

BA71ΔCD2: a new recombinant live attenuated African swine fever virus with cross-protective capabilities (Monteagudo et al., 2017[8])

An article published in the Journal of Virology investigated a new recombinant live attenuated African swine fever virus to be used to develop safer and cross protective vaccines against ASF. The researchers found that the deletion of the viral CD2v (EP402R) gene highly attenuated the virulent BA71 strain in vivo. Inoculation of pigs with the deletion mutant virus BA71ΔCD2 conferred protection not only against lethal challenge with the parental BA71 but also against the heterologous E75 (both genotype I strains). The protection induced was dose-dependent, and the cross-protection observed in vivo correlated with the ability of BA71ΔCD2 to induce specific CD8+ T cells capable of recognising both BA71 and E75 viruses in vitro. Interestingly, 100% of the pigs immunised with BA71ΔCD2 also survived lethal challenge with Georgia 2007/1, the genotype II strain of ASFV currently circulating in continental Europe. The authors conclude that these results would open new avenues to design ASFV cross-protective vaccines, which would be essential to fight ASFV in areas where the virus is endemic and where multiple viruses are circulating.

No evidence for long-term carrier status of pigs after African swine fever virus infection (Petrov et al., 2018[9])

An article published in Transboundary and Emerging Diseases describes the assessment of a potential ASF virus (ASFV) carrier state of 30 pigs which were allowed to recover from experimental infection with ASFV "Netherlands'86" prior to exposure to six healthy sentinel pigs for more than 2 months. Virological and serological investigations were routinely conducted on blood and swab samples throughout the whole trial. At the end of the experiment, necropsy of all animals was performed, and viral persistence and distribution were assessed. Upon infection, a wide range of clinical and pathomorphological signs were observed. After an initial acute phase in all experimentally inoculated pigs, 66.6% recovered completely and seroconverted. However, viral genome was detectable in blood samples for up to 91 days. Lethal outcomes were observed in 33.3% of the pigs with both acute and prolonged courses. No ASFV transmission occurred over the whole in-contact phase from survivors to sentinels. Similarly, infectious ASFV was not detected in any of the tissue samples from ASFV convalescent and in-contact pigs. The authors assumed, based on these findings, that the suggested role of ASFV survivors is overestimated and has to be reconsidered thoroughly for future risk assessments.

First Oral Vaccination of Eurasian Wild Boar Against African Swine Fever Virus Genotype II (Barasona et al., 2019[10])

An article published in Frontiers in Veterinary Science reported of a promising vaccine against ASF virus in wild boar when given by oral administration. In the European Union, wild boar (Sus scrofa) is the most severely affected host. The main reasons for the unprecedented and constant spread of ASF in Europe are trade activities, the continuous movement of infected-wild boar populations among regions and the lack of vaccine to prevent ASF infection. In this study, the authors demonstrated that oral immunisation of wild boar with a non-haemadsorbing, attenuated ASF virus of genotype II

8 Monteagudo, P. L., Lacasta, A., López, E., Bosch, L., Collado, J., Pina-Pedrero, S., ... & Bustos, M. J. (2017). BA71ΔCD2: A new recombinant live attenuated African swine fever virus with cross-protective capabilities. *Journal of Virology*, JVI-01058.

9 Petrov, A., Forth, J. H., Zani, L., Beer, M., & Blome, S. (2018). No evidence for long-term carrier status of pigs after African swine fever virus infection. *Transboundary and Emerging Diseases*.

10 Barasona, J. A., Gallardo, C., Cadenas-Fernández, E., Jurado, C., Rivera, B., Rodríguez-Bertos, A., ... & Sánchez-Vizcaíno, J. M. (2019). First oral vaccination of Eurasian wild boar against African swine fever virus genotype II. *Frontiers in veterinary science*, 6, 137.

isolated in Latvia in 2017 (Lv17/WB/Rie1) conferred 92% protection against challenge with a virulent ASF virus isolate (Arm07). Further studies should assess the safety of repeated administration and overdose, characterise long-term shedding and verify the genetic stability of the vaccine virus to confirm if Lv17/WB/Rie1 could be used for free-ranging wild boar in ASF control programmes.

Epidemiological considerations on African swine fever in Europe 2014–2018 (Chenais et al., 2019[11])

An article published in Porcine Health Management made some epidemiological considerations on ASF in the European Union (EU), following the introduction of the disease in 2014. Until the detection of the first case inside the EU, infections in the current epidemic were mainly seen among pig farms with generally low biosecurity, and with incidental spill over to the wild boar population. In the EU, however, the infection survived locally in the wild boar population independently from outbreaks in domestic pigs, with a steady and low prevalence. Apart from the wild boar population and the habitat, the current epidemic recognises humans as being mainly responsible for both long distance transmission and virus introduction in the domestic pig farms. This underlines the importance to include social science when planning ASF-prevention, –control, or -eradication measures. Based on experiences, knowledge and data gained from the current epidemic this review highlights some recent developments in the epidemiological understanding of ASF, especially concerning the role of wild boar and their habitats in ASF epidemiology. In this regard, the qualities of three epidemiological traits: contagiousness, tenacity, and case fatality rate, and their impact on ASF persistence and transmission are especially discussed.

African swine fever virus evasion of host defences (Dixon et., 2019[12])

In this article, published in Virus Research, the authors reviewed available scientific knowledge about how ASF virus interacts with and modulates the host's responses to infection. The virus' long double-stranded DNA genome codes for more than 160 proteins of which many are non-essential for replication in cells but can have important roles in evading the host's defences. This review considered knowledge of the pathways targeted by ASFV and the mechanisms by which these are inhibited. The impact of deleting single or multiple ASFV genes on virus replication in cells and infection in pigs is summarised providing information on strategies for rational development of modified live vaccines.

Transmission of African Swine Fever Virus via carrier (survivor) pigs does occur (Eblé et al., 2019[13])

An article published in Veterinary Microbiology investigated whether ASF carrier pigs that had completely recovered from an acute infection with ASFV Netherlands '86 could transmit the disease to naive pigs by direct contact transmission. For this, pigs that had survived an ASFV infection, had recovered from disease, and had become carriers of ASFV were used. These clinically healthy carriers were put together one-by-one with naive contact pigs. Two of the twelve contact pigs developed an acute ASFV infection. Using the results of the experiment, the authors quantified the transmission parameters βcarrier (0.039/day) and Tcarrier (25.4 days). With the survival rate of 0.3 for the used ASFV isolate, these parameter values translate into the contribution of carriers to R_0 in groups of pigs being 0.3. Further, naive contact pigs were placed in an ASFV contaminated environment. There, no contact infections were observed. Based on these findings, the authors concluded that clinically healthy carriers can be a source of acute new infections, which can contribute to the persistence of ASFV in swine populations. The estimates that are provided in the article can be used for modelling of transmission in domestic pigs and, in part, for modelling transmission in wild boar.

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11 Chenais, E., Depner, K., Guberti, V., Dietze, K., Viltrop, A., & Ståhl, K. (2019). Epidemiological considerations on African swine fever in Europe 2014–2018. *Porcine health management*, 5(1), 6.

12 Dixon, L. K., Islam, M., Nash, R., & Reis, A. L. (2019). African swine fever virus evasion of host defences. *Virus research* 266, 25-33.

13 Eblé, P. L., Hagenaars, T. J., Weesendorp, E., Quak, S., Moonen-Leusen, H. W., & Loeffen, W. L. A. (2019). Transmission of African Swine Fever Virus via carrier (survivor) pigs does occur. *Veterinary microbiology*, 108345.

Research gap analysis on African swine fever (EFSA, 2019[14])

The European Food Safety Authority (EFSA) conducted, under the request of the European Commission, an online survey to identify the most significant knowledge gaps in the prevention and control of ASF being perceived by EU Veterinary services and other stakeholders involved in pig production and wild boar management. The respondents were asked to identify the major research needs in order to improve short-term ASF risk management. Four major gaps were identified: 'wild boar', 'ASF virus (ASFV) survival and transmission', 'biosecurity' and 'surveillance'. In particular, the respondents stressed the need for better knowledge on wild boar management and surveillance, and improved knowledge on the possible mechanism for spread and persistence of ASF in wild boar populations. They indicated the need for research on ASFV survival and transmission from the environment, different products such as feed and feed materials, and potential arthropod vector transmission. In addition, several research topics on biosecurity were identified as significant knowledge gaps and the need to identify risk factors for ASFV entry into domestic pig holdings, to develop protocols to implement specific and appropriate biosecurity measures, and to improve the knowledge about the domestic pig–wild boar interface. Potential sources of ASFV introduction into unaffected countries need to be better understood by an in-depth analysis of the possible pathways of introduction of ASFV with the focus on food, feed, transport of live wild boars and human movements. Finally, research on communication methods to increase awareness among all players involved in the epidemiology of ASF (including truck drivers, hunters and tourists) and to increase compliance with existing control measures was also a topic mentioned by all stakeholders.

Epidemiological evaluation of Latvian control measures for African swine fever in wild boar on the basis of surveillance data (Schulz et al., 2019[15])

An article published on Scientific Reports evaluated the effectiveness of the ASF control measures implement by Latvia on wild boars. Wild boar population infected with ASF constitutes a constant threat to commercial pig farms and therefore to the economy of the affected country, but the intensive measures that several countries put in place with the aim to reduce wild boar population densities seems not to be able to stop the further spread of the disease. In addition, there are still substantial knowledge gaps regarding the epidemiology of the disease. To identify risk factors for a higher probability of a wild boar sample being virological or serological positive, comprehensive statistical analyses were performed based on Latvian surveillance data. Using a multivariable Bayesian regression model, the effects of implemented control measures on the proportion of hunted or found dead wild boar that are positive or on the estimated virus prevalence were evaluated. None of the control measures applied in Latvia showed a significant effect on the relevant target figure. Also, the estimated periodic prevalence of wild boar that had tested ASF positive by PCR appeared to remain unaffected over time. Therefore, the author suggested that there is an urgent need to reconsider the implemented control measures and raise the question whether an endemic situation of ASF in wild boar is reversible.

Architecture of African swine fever virus and implications for viral assembly (Wang et al., 2019[16])

An article published in Science shed light on the architecture of the African swine fever virus (ASFV). Using an optimised image reconstruction strategy, the authors solved the ASFV capsid structure up to 4.1-angstroms, which is built from 17,280 proteins, including one major (p72) and four minor capsid proteins (M1249L, p17, p49 and H240R), organized into pentasymmetrons and trisymmetrons. The

14 European Food Safety Authority (EFSA), Álvarez, J., Bicout, D., Boklund, A., Bøtner, A., Depner, K., ... & Viltrop, A. (2019). Research gap analysis on African swine fever. *EFSA Journal*, 17(8), e05811.

15 Schulz, K., Oļševskis, E., Staubach, C., Lamberga, K., Seržants, M., Cvetkova, S., ... & Sauter-Louis, C. (2019). Epidemiological evaluation of Latvian control measures for African swine fever in wild boar on the basis of surveillance data. *Scientific reports*, 9(1), 4189.

16 Wang, N., Zhao, D., Wang, J., Zhang, Y., Wang, M., Gao, Y., ... & Wang, X. (2019). Architecture of African swine fever virus and implications for viral assembly. *Science*, eaaz1439.

atomic structure of the p72 informs putative conformational epitopes, distinguishing ASFV from other nucleocytoplasmic large DNA viruses (NCLDV). The minor capsid proteins form a complicated network below the outer capsid shell, stabilizing the capsid by holding adjacent capsomers together. Acting as core organizers, 100-nm long M1249L proteins run along each edge of trisymmetrons bridging two neighbouring pentasymmtrons and form extensive intermolecular networks with other capsid proteins, driving the formation of the capsid framework. The authors concluded that these structural details unveil the basis of capsid stability and assembly, opening up new avenues for ASF vaccine development.

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 bovine TB;
- Develop novel and improved tools to control bovine TB;
- Serve as a communication and technology sharing gateway for the global bovine TB 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 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 is planned to be held on the side of the 7th International Conference on *Mycobacterium bovis* that will be held in Galway (Ireland) in June 2020.

DISCONTOOLS

The information on bTB was updated in August 2016. Below follows an extraction of identified gaps in the field of diagnostics, vaccines and pharmaceuticals. Other knowledge gaps and more information are available at www.discontools.eu.

Diagnostics

The predominant method for diagnosis of bTB in live cattle is the tuberculin skin test, consisting of an intradermal injection of a purified protein derivatives from a culture of M. bovis (bovine PPD), or alternatively, to increase specificity, the comparison of reactions induced after injection of bovine

and avian PPD (the latter produced from a culture of M. avium). IFN-γ release assays (IGRAs) have also been developed and are being increasingly applied. When used in combination with skin tests, overall sensitivity is increased.

Tuberculins are largely undefined and difficult to produce and standardise (e.g. BCL3 facilities are required, including animal facilities to perform guinea pig potency assays). Therefore, the development of defined skin test reagents based on specific M. bovis antigens would be beneficial to overcome tuberculin limitations.

Several sero-diagnostic tests have been developed or are presently being developed but generally lack sensitivity compared to the IGRA and skin test, but have been usefully applied in some wildlife and domestic animal species (e.g. deer or South American Camelids).

Better tests that are rapid, specific and simple are needed for live animals, particularly for cattle in developing countries, and for wildlife species.

Vaccines

At present, the only potentially available vaccine is BCG, which is a live attenuated strain of M. bovis used for humans since the 1920s. Studies with BCG showed variable efficacy in cattle at population and individual animal levels. Although BCG can prevent the development of pathology/bacillary persistence in a proportion of animals, as in humans, in most studies BCG vaccination did not prevent infection but reduced the number and severity of pathology, and thus likely reduced transmission. The use of BCG will however compromise specificities of tuberculin-based tests and the development of DIVA (Differentiating Infected from Vaccinated Animals) tests for cattle is essential. The commercial potential for effective vaccines is high in some countries where bTB remains a problem.

Improved vaccines for cattle are under active development based on genetically modified BCG or M. bovis, DNA, protein or virally vectored subunits, used stand-alone or in conjunction with BCG. Non-sensitising vaccines would overcome the problem of skin test sensitisation associated with BCG-based strategies.

BCG vaccines may reduce M. bovis in wildlife reservoirs and an injectable vaccine has been licensed for use in badgers in UK. The further development of delivery systems for the application of vaccines in wildlife is needed.

Pharmaceuticals

Antimicrobial treatment is not applicable for bTB control in livestock.

Recent developments

Genetic evaluation for bovine tuberculosis resistance in dairy cattle (Banos et al., 2017[17])

This paper, published in the Journal of Dairy Science, presented a genetic evaluation for bTB resistance in dairy cattle. Calculations were based on British national data covering individual animal tuberculin skin test results, post-mortem examination, animal movement and location information, production history, and pedigree records. Only Holstein cows with identified sires in herds with bTB breakdowns (new herd incidents) occurring between the years 2000 and 2014 were considered. Resistance estimated heritability appeared to have low heritability but high repeatability. In addition, the analyses showed that correlations of genetic evaluations for bTB with other traits in the current breeding goal were mostly not different from zero. Correlation with the UK Profitable Lifetime Index was moderate, significant, and favourable. The authors concluded that the study demonstrated the feasibility of a national genetic evaluation for bTB resistance, suggesting that selection for enhanced resistance would

17 Banos, G., Winters, M., Mrode, R., Mitchell, A. P., Bishop, S. C., Woolliams, J. A., & Coffey, M. P. (2017). Genetic evaluation for bovine tuberculosis resistance in dairy cattle. *Journal of Dairy Science*, 100(2), 1272-1281.

have a positive effect on profitability and no antagonistic effects on current breeding goal traits.

Proteomic characterisation of bovine and avian purified protein derivatives and identification of specific antigens for serodiagnosis of bovine tuberculosis (Infantes-Lorenzo et al., 2017[18])

In this article, published in Clinical Proteomics, the authors carried out a proteomic characterisation of bovine purified protein derivative (bPPD), avian purified protein derivative (aPPD) and an immunopurified subcomplex from bPPD called P22. Aim of the study was to better identify qualitative and quantitative characteristics of these products, and to identify proteins contributing to cross-reactivity among these three products in tuberculosis diagnosis. A total of 456 proteins were identified in bPPD, 1019 in aPPD and 118 in P22; of these, 146 were shared by bPPD and aPPD, and 43 were present in all three preparations. Candidate proteins that may cause bPPD-aPPD cross-reactivity were identified based on protein abundance and antigenic propensity. Serum reactivity experiments indicated that P22 may provide greater specificity than bPPD with similar sensitivity for ELISA-type detection of antibodies against M. tuberculosis complex. The authors suggested that P22 may be an alternative to bPPD for serodiagnosis of bovine tuberculosis, since it shares fewer proteins with aPPD than bPPD does, reducing risk of cross-reactivity with anti-M. avium antibodies.

Identification of novel antigens recognized by serum antibodies in bovine tuberculosis (Lyashchenko et al., 2017[19])

An article published in Clinical and Vaccine Immunology describes the investigations of novel antigens recognised by serum antibodies in bovine tuberculosis (bTB) that might improve the current bTB testing. The authors screened a panel of 101 recombinant proteins, including 10 polyepitope fusions, by a multiantigen print immunoassay (MAPIA) with well-characterised serum samples collected serially from experimental or naturally M. bovis infected cattle. A novel set of 12 sero-reactive antigens was established. The evaluation of the selected proteins in the dual-path platform (DPP) assay showed that the highest diagnostic accuracy (~95%) was achieved with a cocktail of five best-performing antigens. The authors concluded that these results demonstrate the potential for development of an improved and more practical sero-diagnostic test for bovine bTB.

Transmission of tuberculosis caused by Mycobacterium caprae between dairy sheep and goats (Vidal et al., 2018[20])

Several cases of tuberculosis (TB) in caprine, caused either by Mycobacterium bovis or Mycobacterium caprae, have been recently reported in a number of countries being involved in tuberculosis eradication campaigns. M. caprae transmission and its possible role in hindering TB eradication plans are still unclear. This article describes the investigation on the transmission of the infection between sheep and goats conducted during a tuberculosis outbreak in a caprine/ovine dairy mixed herd. Tuberculin skin test positive goats and ewes were euthanised and subsequent post-mortem investigations were performed. M. caprae (spoligotype profile SB0157) was isolated from tuberculous lesions detected in both sheep and goats. The researchers found evidences of direct transmission of the infection between both species, elucidating that not only goats but also sheep may act as domestic reservoirs of TB, and could thus compromise the eradication of TB in cattle. The authors concluded that these results would have implications for animal TB epidemiology and public health risk management.

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18 Infantes-Lorenzo, J. A., Moreno, I., de los Ángeles Risalde, M., Roy, Á., Villar, M., Romero, B., ... & Gortázar, C. (2017). Proteomic characterisation of bovine and avian purified protein derivatives and identification of specific antigens for serodiagnosis of bovine tuberculosis. *Clinical Proteomics*, 14(1), 36.

19 Lyashchenko, K. P., Grandison, A., Keskinen, K., Sikar-Gang, A., Lambotte, P., Esfandiari, J., ... & Vordermeier, H. M. (2017). Identification of novel antigens recognized by serum antibodies in bovine tuberculosis. *Clinical and Vaccine Immunology*, CVI-00259.

20 Vidal, E., Grasa, M., Perálvarez, T., Martín, M., Mercader, I., & de Val, B. P. (2018). Transmission of tuberculosis caused by Mycobacterium caprae between dairy sheep and goats. *Small Ruminant Research*, *158*, 22-25.

Interleukin 8 and Pentaxin (C-Reactive Protein) as Potential New Biomarkers of Bovine Tuberculosis (Gao et al., 2019[21])

An article, published in the Journal of Clinical Microbiology, investigated the use of interleukin 8 (IL8) and pentaxin (C-Reactive protein) as potential new biomarkers of bTB. During the early stage of infection, more than 15% of M. bovis-infected cattle shed mycobacteria through nasal secretions, which can be detected by nested PCR. To compare the differences in the protein profiles of M. bovis-infected cattle that were nested PCR positive (bTBPCR-P) and M. bovis-infected cattle that were nested PCR negative (bTBPCR-N) and to screen for biomarkers that will facilitate the early and accurate detection of bTB, the authors investigated the protein expression profiles of serum and plasma deriving from bovine purified protein derivative (PPD-B)-stimulation among bTBPCR-P (n = 20), bTBPCR-N (n = 20), and uninfected cattle (NC; n = 20) by iTRAQ labelling coupled with two-dimensional liquid chromatography-tandem mass spectrometry (iTRAQ-2D LC-MS/MS). After comprehensive analysis, 15 putative differentially expressed serum proteins and 15 plasma proteins were selected for validation by parallel reaction monitoring (PRM) with the same cohort used in the iTRAQ analysis. Four serum and five PPD-B-stimulated proteins were confirmed in follow-up enzyme-linked immunosorbent assays. The IL-8 produced under stimulation by PPD-B- displayed the potential to differentiate M. bovis-infected cattle from NC, with an area under the curve (AUC) value of 0.9662, while PPD-B-stimulated C-reactive protein (CRP) displayed the potential to differentiate bTBPCR-P from bTBPCR-N, with an AUC value of 1.00. Finally, double-blind testing with 244 cattle indicated that the PPD-B-stimulated IL-8 test exhibited good agreement with traditional tests ($\kappa > 0.877$) with a >90% relative sensitivity and a >98% relative specificity; the PPD-B-stimulated CRP test displayed good agreement with nested PCR ($\kappa = 0.9117$), with an observed 94% relative sensitivity and 97% relative specificity. Therefore, the author suggested that the PPD-B-stimulated IL-8 and CRP tests could be used to detect bTB and to differentiate bTBPCR-P from bTBPCR-N.

Nilotinib: A Tyrosine Kinase Inhibitor Mediates Resistance to Intracellular Mycobacterium Via Regulating Autophagy (Hussain et al., 2019[22])

This article, published in Cells, investigated the pharmacological action of nilotinib, a tyrosine kinase inhibitor that has been studied extensively in various tumor models, against M. bovis and M.avium subspecies paratuberculosis (MAP). Although M. bovis and MAP have distinct tissue tropism, both of them infect, reside, and replicate in mononuclear phagocytic cells of the infected host. Autophagy is an innate immune defence mechanism for the control of intracellular bacteria, regulated by diverse signalling pathways. Results from the study demonstrated that nilotinib significantly inhibited the intracellular survival and growth of M. bovis and MAP in macrophages by modulating host immune responses. Nilotinib-induced autophagic degradation of intracellular mycobacterium occurred via the inhibition of PI3k/Akt/mTOR axis mediated by abelson (c-ABL) tyrosine kinase. In addition, it was observed that nilotinib promoted ubiquitin accumulation around M. bovis through activation of E3 ubiquitin ligase parkin. From in-vivo experiments, the authors found that nilotinib effectively controlled *M. bovis* growth and survival through enhanced parkin activity in infected mice. Altogether, the data showed that nilotinib regulates protective innate immune responses against intracellular mycobacterium, both in-vitro and in-vivo, its exploitation as a novel therapeutic remedy for the control of *M. bovis* and MAP infections could be considered.

21 Gao, X., Guo, X., Li, M., Jia, H., Lin, W., Fang, L., ... & Xin, T. (2019). Interleukin 8 and pentaxin (C-reactive protein) as potential new biomarkers of bovine tuberculosis. *Journal of clinical microbiology*, 57(10), e00274-19.

22 Hussain, T., Zhao, D., Shah, S. Z. A., Sabir, N., Wang, J., Liao, Y., ... & Yang, L. (2019). Nilotinib: A Tyrosine Kinase Inhibitor Mediates Resistance to Intracellular Mycobacterium Via Regulating Autophagy. *Cells*, 8(5), 506.

A defined antigen skin test for the diagnosis of bovine tuberculosis (Srinivasan et al. 2019[23])

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 that has equal or superior performance over the extant standard tuberculin surveillance test. Together with the ease of chemical synthesis, quality control, and lower burden for regulatory approval compared with recombinant antigens, the results of these studies showed that the DST considerably improves a century-old standard and enables the development and implementation of critically needed surveillance and vaccination programs to accelerate bTB control.

3. Brucellosis

Global network

Under the STAR-IDAZ project, an expert group on brucellosis had been 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 will have a presentation at the 2019 International Brucellosis Society Meeting, that will be 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 will be to present 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.

DISCONTOOLS

The updated information for Brucellosis was published in June 2018. Below follows an extraction of identified gaps in the field of diagnostics, vaccines and pharmaceuticals. Other knowledge gaps and more information are available at www.discontools.eu.

Diagnostics

Many commercial diagnostic kits are available worldwide but, although costs of tests are generally competitive, they are out of reach for many areas in Africa or Asia. Almost all kits require cold storage, and this may be a problem in some resource poorer regions.

Several methods such as the Complement Fixation test, iELISA, cELISA, a fluorescence polarisation assay, Rose Bengal test and brucellin skin tests are available for the detection of B. abortus, B. melitensis or B. suis. Penside serological assays, such as lateral flow assays, are in development but are not yet in validation trials.

Culture of the organism is the only unequivocal diagnostic method and is especially important in non-endemic areas, but this is slow, expensive and presents significant risks to diagnosticians. More effective selective enrichment and culture media are required. Conventional typing is difficult and poses reproducibility problems and could be advantageously replaced by molecular methods.

23 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.

There are no commercially available PCR kits that claim to detect Brucella DNA. Several PCR protocols have been optimised for sensitivity and specificity under laboratory conditions but are insufficiently sensitive on accessible clinical material. These methods are currently expensive although cheaper alternatives are in development.

Information is lacking on the performance of serological tests in swine, camelids, yaks, water buffaloes and wildlife. All serological tests need validation according to local conditions and the specific animal host.

Vaccines

Vaccines are only available against B. abortus (cattle) and B. melitensis or B. ovis (small ruminants) infections. There have been several attempts to produce subcellular or DNA based vaccines, but none are as practical and/or effective as the current vaccines. The effective vaccines are currently live attenuated strains.

There is a need for new vaccines that are more protective, able to generate immune responses easily differentiable from those of infected animals (DIVA assays are required) and less pathogenic for livestock (not abortifacient) and handlers. More stable and more affordable vaccines are also required.

Pharmaceuticals

Therapy is seldom used in animals. For human brucellosis, more efficacious and cheaper antibiotics would be valued that avoid parental administration, have a shorter administration period, avoid relapses and make treatment more affordable.

Recent developments

In vitro synergistic effects of a short cationic peptide and clinically used antibiotics against drug-resistant isolates of Brucella melitensis (Azad et al., 2017[24])

Although this paper focussed on human therapy, the presented results might be of interest for animal health as well. In this article, Azad and colleagues described the evaluation of the antimicrobial effects of the CM11 peptide alone and combined with common antibiotics against drug-resistant isolates of B. melitensis. The authors evaluated antibiotic susceptibility pattern from pathogenic samples of B. melitensis by E-test and evaluated the synergistic reaction of the peptide with selected antibiotics using a chequerboard procedure. Synergic effect was observed for streptomycin and co-trimoxazole in combination with the peptide while ciprofloxacin and rifampin showed partial synergy and additive effect, respectively. The authors concluded that using antibiotic-CM11 combination, their effective dose can be reduced particularly for drug-resistant isolates.

Brucellosis: improved diagnostics and vaccine insights from synthetic glycans (Bundle & McGiven, 2017[25])

Detection of antibodies to the Brucella bacterial cell wall O-polysaccharide (OPS) component of smooth lipopolysaccharide is used in diagnosis of this disease, and the same molecule contributes important protective efficacy to currently deployed veterinary whole-cell vaccines. Thus, the most protective brucellosis vaccines compromise serodiagnosis, making DIVA not possible. Recent studies on the chemical structure of Brucella OPS revealed it being a block copolymer of two oligosaccharide

24 Azad, Z. M., Moravej, H., Fasihi-Ramandi, M., Masjedian, F., Nazari, R., Mirnejad, R., & Moghaddam, M. M. (2017). In vitro synergistic effects of a short cationic peptide and clinically used antibiotics against drug-resistant isolates of Brucella melitensis. *Journal of Medical Microbiology*, 66(7), 919-926.

25 Bundle, D. R., & McGiven, J. (2017). Brucellosis: improved diagnostics and vaccine insights from synthetic glycans. *Accounts of Chemical Research*, 50(12), 2958-2967.

sequences; there is thus an opportunity to use unique oligosaccharides in serodiagnostic tests for the disease. These oligosaccharides show excellent sensitivity and specificity compared with the native polymer used in current commercial tests and have the added advantage of assisting discrimination between brucellosis and infections caused by several bacteria with OPS that share some structural features with those of Brucella. Moreover, these synthetic antigens provide an opportunity to create a polysaccharide-protein conjugate vaccine with DIVA properties. In this article, immunisation of mice showed that antibodies to the Brucella A antigen could be developed without reacting in a diagnostic test based on the M antigen. A conjugate vaccine of this type could readily be developed for use in humans and animals. Considering the advances in chemical methods and maturation of modern methods of bacterial engineering, the authors expects that these principles could be applied to the development of an inexpensive and cost-effective vaccine to combat endemic brucellosis in animals.

A novel real-time PCR assay for specific detection of Brucella melitensis (Kaden et al., 2017[26])

An article published in BMC Infectious Diseases describes the setting up of a species-specific real-time PCR for the detection of all biovars of Brucella melitensis, which could be used routinely in diagnostic laboratories. Using all available genomes in the public database of Brucella (N=96) including all complete genomes of B. melitensis (N=17), the authors designed a Brucella melitensis real-time PCR assay. The assay was validated with a collection of 37 Brucella species reference strains, 120 B. melitensis human clinical isolates, and 45 clinically relevant non-B. melitensis strains. This new real-time PCR method showed a high specificity (100%) and a high sensitivity (1.25 GE/µl) and has been already implemented in the laboratories of four governmental authorities across Sweden.

Brucellosis vaccines based on the open reading frames from genomic island 3 of Brucella abortus (Gómez et al., 2018[27])

The control of brucellosis in cattle through immunisation with live attenuated B. abortus S19 and RB51 strains has been associated with safety issues in animals and humans. In this review, published in Vaccine, the authors present the state of the art of the development of new DNA vaccines, which have shown effectiveness and a good safety profile. Some antigenic candidates for vaccine design against brucellosis (mainly DNA vaccines) have been obtained from genomic island 3 (GI-3) of B. abortus, which encodes several open reading frames (ORFs) involved in the intracellular survival and virulence of this pathogen. The data presented about immunogenicity and protection conferred by these DNA vaccines in a murine model suggests that some of them could be safe and effective vaccine candidates to prevent B. abortus infection. Besides these vaccine candidates, the author highlighted the importance of GI-3 as source to develop promising vaccines, mainly DNA vaccines, in the prevention of B. abortus infection.

Rapid visual isothermal nucleic acid-based detection assay of Brucella species by polymerase spiral reaction (Das et al., 2018[28])

In an article published in the Journal of Applied Microbiology, the authors studied the development of polymerase spiral reaction (PSR) for rapid, sensitive and specific detection of Brucella spp. Polymerase spiral reaction assay was developed using specifically designed primers targeting the conserved multicopy IS711 gene of Brucella spp. The lower limit of detection of PSR was 11.8 fg and conventional PCR was 1.18 pg of Brucella abortus genomic DNA. Thus, PSR was found to be 100-fold more sensitive than conventional PCR and was comparable to real-time PCR. The specificity of PSR was tested with

26 Kaden, R., Ferrari, S., Alm, E., & Wahab, T. (2017). A novel real-time PCR assay for specific detection of Brucella melitensis. *BMC Infectious Diseases*, *17*(1), 230.

27 Gómez, L., Alvarez, F., Betancur, D., & Oñate, A. (2018). Brucellosis vaccines based on the open reading frames from genomic island 3 of Brucella abortus. Vaccine, 36(21), 2928-2936.

28 Das, A., Kumar, B., Chakravarti, S., Prakash, C., Singh, R. P., Gupta, V., ... & Shrinet, G. (2018). Rapid visual isothermal nucleic acid-based detection assay of Brucella species by polymerase spiral reaction. *Journal of applied microbiology*, 125(3), 646-654.

other non-Brucella bacteria and also with some bacterial and viral pathogens causing abortions. The assay was found to be specific as it did not detect any putative pathogens other than Brucella spp. Fifty-six clinical samples from suspected brucellosis cases (aborted foetal stomach content) were screened with PSR to validate the applicability of the test to detect Brucella DNA. The same samples were also screened with conventional PCR and real-time PCR. Of 56 samples, 25 samples were found to be positive with both PSR as well as real-time PCR, whereas only 20 samples were found positive with conventional PCR. The results of this study indicated that the PSR assay is a simple, rapid, sensitive and specific method for the detection of Brucella spp. that may improve diagnostic potential in clinical laboratories or can be used at diagnostic laboratories with minimal infrastructure. The authors conceded that the PSR assay, because of its simplicity and low cost, can be preferred to other molecular methods in the diagnosis of infectious diseases.

The key role of c-Fos for immune regulation and bacterial dissemination in Brucella infected macrophage (Hop et al., 2018[29])

An article published in Frontiers in Cellular and Infection Microbiology investigated the role of the cellular oncogene c-Fos (c-Fos) in immune regulation and bacterial dissemination in Brucella infected macrophage. c-Fos is a component of activator protein 1 (AP1), a master transcriptional regulator of cells. The suppression of c-Fos signalling by siRNA treatment resulted in significant induction of TLR4, which subsequently activates p38 and ERK1/2 mitogen-activated protein kinases (MAPKs) and enhances F-actin polymerisation, leading to an increase in B. abortus phagocytosis. During B. abortus infection, c-Fos signalling is induced, which activates the downstream innate-immunity signalling cascade for bacterial clearance. The inhibition of c-Fos signalling led to increased production of interleukin 10 (IL-10), which partially suppressed lysosome-mediated killing, resulting in increased survival of B. abortus inside macrophages. The authors presented evidence of the regulatory role played by the c-Fos pathway in proliferation during B. abortus infection; however, this was independent of the anti-Brucella effect of this pathway. In addition, they found that the essential contribution of c-Fos/TRAIL is to infected-cell necrosis, which is a key event in bacterial dissemination. These data provided the mechanism via which c-Fos participates in host defence mechanisms against Brucella infection and in bacterial dissemination by macrophages.

Development of an auxotrophic, live-attenuated Brucella suis vaccine strain capable of expressing multimeric GnRH (Smith et al., 2019[30])

An article published in Vaccine preliminary reported on the development of a live-attenuated B. suis vaccine that could be employed to deliver heterologous antigens to control the diseases in swine populations. Feral swine cost around $1.5 billion each year in agricultural, environmental, and personal property damages. They are also the most widespread carriers of the zoonotic disease brucellosis, which threatens both livestock bio-security and public health. Currently, there is no approved vaccine against brucellosis in pigs. An attenuated vaccine strain provided significant protection against B. suis challenge in mice. Leucine auxotrophy in the vaccine strain allowed the over-expression of heterologous antigens without the use of antibiotic resistant markers. Vaccinated mice showed the development of antibodies against expressed antigen. Further evaluation is required to assess its ability to cause infertility using the mouse model prior to further testing for use as a tool for feral swine population and disease control.

29 Hop, H. T., Arayan, L. T., Huy, T. X. N., Reyes, A. W. B., Vu, S. H., Min, W., ... & Kim, S. (2018). The key role of c-Fos for immune regulation and bacterial dissemination in Brucella infected macrophage. *Frontiers in Cellular and Infection Microbiology*, 8, 287.

30 Smith, G. P., Jain-Gupta, N., Alqublan, H., Dorneles, E. M. S., Boyle, S. M., & Sriranganathan, N. (2019). Development of an auxotrophic, live-attenuated *Brucella suis* vaccine strain capable of expressing multimeric GnRH. *Vaccine*, 37(7), 910-914.

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, 23 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 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. SIRCAH is organising a satellite workshop on the side of the GFRA Scientific Meeting that will be held in Bangkok in October 2019, for the validation of the roadmaps by the experts. Once validated, the roadmaps will be published on the STAR-IDAZ IRC website.

DISCONTOOLS

The information on FMD was last updated in May 2015. Gaps in the field of diagnostics, vaccines and pharmaceuticals are presented below. Other knowledge gaps and more information are available at www.discontools.eu.

Diagnostics

Commercialisation of diagnostics for FMD is constrained by lack of resources in developing countries and uncertain demand in developed ones that are mostly FMD free. Diagnostics for FMD are only available from a small number of commercial suppliers. The main reagents used can only be obtained

from the OIE/FAO Reference Laboratory in Pirbright or produced for local use in National or Regional Laboratories. The main commercial reagents include serology kits for NSP testing, while commercial kits for structural antibodies are highly limited and often based on rather old methods, such as the liquid phase blocking ELISA. New commercialised tests for antibodies and for virus and tests that can type across all the variants within serotypes have recently become available from the National and OIE Reference Laboratory for FMD in Brescia, Italy.

Faster diagnostics and field pen side tests are required, along with the development of more effective and specific differential tests. Lateral flow devices for virus detection are now available but not yet for distinguishing between all seven serotypes. Prototypes exist for portable units that detect viral RNA with high sensitivity. The development of improved rapid and inexpensive diagnostic assays would assist in surveillance. The lack of sufficient panels for test validation across all serotypes and species is a constraint.

Assays to distinguish between vaccinated and infected animals with improved sensitivity are available, but the lack of knowledge about virus transmission and persistence in vaccinated populations creates uncertainty about reliability of these tests to detect undisclosed infection.

Vaccines

Support for fundamental immunology and for animal studies is essential. Current vaccines are efficient provided that they are applied before exposure to live virus (at least 1 week before exposure), that the vaccine strain has been carefully selected to match the outbreak strain, that sufficient amount of intact antigen is included in the vaccine and that the vaccine is of good quality. There are disadvantages with the current vaccines, which include the dangers inherent in their large-scale production from virulent virus and the heat labile nature of the vaccine, necessitating provision of a cold chain and the short duration of protection elicited. Not all strains of FMD virus are covered fully by the limited number of vaccine strains commercially available, and new variants emerge periodically.

The need to either know the antigenic characteristics of the outbreak virus strain, or to add multiple antigens to the vaccine, increases the costs of vaccination significantly. Lack of knowledge on circulating isolates in endemic regions may affect the efficacy of vaccination campaigns due to incorrect selection of the antigens in endemic settings. In addition, the need for regular booster vaccinations is a major constraint to maintain protective levels of immunity.

In the USA, adenovirus vectored vaccines have become commercially available for some serotypes with a reduced risk for FMD virus escape during production or from incomplete inactivation. Another promising line of research is the development of recombinant empty capsids, which may have enhanced stability and could be produced without the need to handle live FMD virus.

Pharmaceuticals

There may be some potential for the use of antivirals in FMD control but there would be considerable challenges in both developing and licensing such products. Some compounds with in vitro antiviral activity have been identified but problems such as safety, oral effectiveness and avoidance of virus resistance remain to be overcome.

Recent developments

Global foot-and-mouth disease research update and gap analysis: 1-Overview of Global Status and Research Needs (Knight-Jones et al., 2016[31])

This paper is the first of a series of seven articles that appeared in Transboundary and Emerging

31 Knight-Jones, T. J. D., Robinson, L., Charleston, B., Rodriguez, L. L., Gay, C. G., Sumption, K. J., & Vosloo, W. (2016). Global Foot-and-Mouth Disease Research Update and Gap Analysis: 1-Overview of Global Status and Research Needs. *Transboundary and*

Diseases to provide an update on research and identified research gaps on FMD. The published information was derived from the analyses carried out by the Global Foot-and-Mouth Research Alliance (GFRA), which reviewed all research publications (2011-2015) and actively collected activity updates from 33 FMD research institutes from around the world. This first overview paper provided background information and key findings, while the following ones focused on specific aspects of disease control.

Global foot-and-mouth disease research update and gap analysis: 2-Epidemiology, wildlife and economics (Knight-Jones et al., 2016[32])

This second paper focussed on research related to FMD epidemiology, role of wildlife and economics. The authors highlighted the continued efforts required to develop robust models for use during outbreaks in FMD-free countries, linking epidemiologic and economics models. The evaluation and the setting of targets for vaccine coverage, population immunity and vaccine field efficacy would need more guidance, and methods for seroprevalence studies would need to be improved to obtain more meaningful outputs and allow comparison across studies. Field trials assessing the effectiveness of vaccination in extensive smallholder systems should be performed to determine whether FMD can be controlled with quality vaccines in settings where implementing effective biosecurity is challenging. Studies would need to go beyond measuring only vaccine effects and should extend our knowledge of the impact of FMD and increase our understanding of how to maximise farmer participation in disease control. Where wildlife reservoirs of virus exist, particularly African Buffalo, the way and time of transmission to domestic animals would need to be investigated in order to manage this risk appropriately, considering the impact of control measures on livelihoods and wildlife. For settings where FMD eradication is unfeasible, further ground testing of commodity-based trade is recommended. The authors added that a thorough review of global FMD control programmes, covering successes and failures, would be extremely valuable and could be used to guide other control programmes.

Global foot-and-mouth disease research update and gap analysis: 3-Vaccines (Robinson et al., 2016[33])

This third paper assessed research knowledge gaps in the field of FMDV vaccines so as to identify priority areas for future FMD vaccine research. The authors reported that, while FMD vaccines had little changes over decades, several promising novel FMD vaccine candidates have recently been developed. These included an adenovirus-vectored FMD vaccine, licensed for manufacture and use in the USA, which causes in vivo expression of viral capsids in vaccinated animals. Another promising vaccine candidate comprises stabilised empty FMDV capsids produced in vitro in a baculovirus expression system. Recombinant technologies are also being deployed to improve otherwise conventionally produced inactivated vaccines, (e.g. by creating a chimeric vaccine virus to increase capsid stability and by inserting sequences into the vaccine virus for desired antigen expression). The authors identified enhanced adjuvants, vaccine quality control procedures and predicting vaccine protection from immune correlates as other important areas of ongoing research. The authors concluded that, globally, the degree of independent vaccine evaluation is highly variable, and this is essential for vaccine quality.

Global foot-and-mouth disease research update and gap analysis: 4-Diagnostics (Knight-Jones et al., 2016[34])

This fourth paper focussed on research related to FMD diagnostics and related research gaps. The

Emerging Diseases, 63(S1), 3-13.

32 Knight-Jones, T. J. D., Robinson, L., Charleston, B., Rodriguez, L. L., Gay, C. G., Sumption, K. J., & Vosloo, W. (2016). Global Foot-and-Mouth Disease Research Update and Gap Analysis: 2–Epidemiology, Wildlife and Economics. *Transboundary and Emerging Diseases*, 63(S1), 14-29.

33 Robinson, L., Knight-Jones, T. J. D., Charleston, B., Rodriguez, L. L., Gay, C. G., Sumption, K. J., & Vosloo, W. (2016). Global Foot-and-Mouth Disease Research Update and Gap Analysis: 3-Vaccines. *Transboundary and Emerging Diseases*, 63(S1), 30-41.

34 Knight-Jones, T. J. D., Robinson, L., Charleston, B., Rodriguez, L. L., Gay, C. G., Sumption, K. J., & Vosloo, W. (2016). Global Foot-and-Mouth Disease Research Update and Gap Analysis: 4-Diagnostics. *Transboundary and Emerging Diseases*, 63(S1), 42-48.

authors identified the development of RT-LAMP as an important breakthrough allowing greater use and access to molecular diagnostics. Although PCR can be used to determine virus serotype for certain virus pools, continued progress is needed to cover the global spectrum of FMD viruses. Progress has also been made in the development of pen-side rapid diagnostics, some with the ability to determine serotype. However, further advances in pen-side serotype or strain determination would be important. Novel promising sampling methods were developed (e.g. air sampling and baited ropes, the latter may aid sampling in wildlife and swine). Studies of infrared thermography for the early detection of FMD have not been encouraging, although investigations are ongoing. Multiplex tests have been developed that are able to simultaneously screen for multiple pathogens with similar clinical signs. Crucial for assessing FMDV freedom, tests exist to detect animals that have been infected with FMDV regardless of vaccination status; however, limitations exist, particularly when testing previously vaccinated animals. Novel vaccines are being developed with complementary DIVA tests for this purpose. Research is also needed to improve the current imprecise approaches to FMD vaccine matching. Lastly, the authors concluded that the development of simple, affordable tests would increase access to FMD diagnostics, being of potentially great benefit for the regions with limited laboratory capacity.

Global foot-and-mouth disease research update and gap analysis: 5-Biotherapeutics and disinfectants (Robinson et al., 2016[35])

The fifth paper of the series focussed on the identification of priority areas for future FMD research on biotherapeutics and disinfectants. The authors acknowledged that rapid, short-acting biotherapeutics, aiming either to stimulate a non-specific antiviral state in the animal or to specifically inhibit a part of the viral life cycle, can be useful in case of an outbreak situation. Certain antiviral cytokines have been shown to promote rapid protection against FMD; however, the effects of different immune-modulators appear to vary across species in ways and for reasons that are not yet understood. Major barriers to the effective incorporation of biotherapeutics into control strategies are cost, limited understanding of their effect on subsequent immune responses to vaccines and uncertainty about their potential impact if used for disease containment. Recent research has highlighted the importance of environmental contamination in FMDV transmission. Effective disinfectants for FMDV have long been available, but research is being conducted to further develop methods for quantitatively evaluating their performance under field, or near-field, conditions. The potential environmental contamination deriving from the mass use of disinfectant and mass burial of culled stock should also be considered during outbreak contingency planning.

Global foot-and-mouth disease research update and gap analysis: 6-Immunology (Robinson et al., 2016[36])

The sixth paper targeted FMD immunology, highlighting main research gaps and current research advances on the topic. Continued characterisation of the immune systems of several FMD host species has underpinned substantial advances in knowledge of their interaction with FMDV. Recent studies have shed light on the mechanisms underlying formation of the bovine B- and T-cell responses; there is also a greater understanding of the significance of non-neutralising antibodies during FMDV infection and the interactions of antibody-bound virus with immune cells. This knowledge is directly relevant to vaccine development, as well as understanding protection and cross protection. The authors concluded that, despite ongoing research, significant knowledge gaps remain in the areas of neonatal and mucosal immunity. The impact of maternally derived antibody upon the neonate's ability to respond to FMD vaccination has received some attention, but few firm conclusions can be drawn at this stage, and little is known of the cellular response of young animals in general. The mucosal immune system of FMDV-susceptible species requires continued characterisation, especially if the potential of mucosal vaccine-delivery systems is to be realised for FMD immunisation.

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35 Robinson, L., Knight-Jones, T. J. D., Charleston, B., Rodriguez, L. L., Gay, C. G., Sumption, K. J., & Vosloo, W. (2016). Global Foot-and-Mouth Disease Research Update and Gap Analysis: 5–Biotherapeutics and Disinfectants. *Transboundary and Emerging Diseases*, 63(S1), 49-55.

36 Robinson, L., Knight-Jones, T. J. D., Charleston, B., Rodriguez, L. L., Gay, C. G., Sumption, K. J., & Vosloo, W. (2016). Global Foot-and-Mouth Disease Research Update and Gap Analysis: 6–Immunology. *Transboundary and Emerging Diseases*, 63(S1), 56-62.

Global foot-and-mouth disease research update and gap analysis: 7–Pathogenesis and molecular biology (Robinson et al., 2016[37])

This seventh and last paper focussed on the identification of research gaps and ongoing research on FMD pathogenesis and molecular biology. Several important advances were made in understanding FMD pathogenesis. Investigations found out that FMDV remains in lymph nodes of many recovered animals that otherwise do not appear persistently infected, even in species previously not associated with the carrier state. Whether virus retention helps maintain host immunity and/or virus survival is not known. Studies of FMDV pathogenesis in wildlife have provided insights into disease epidemiology, in endemic and epidemic settings. Many aspects of FMDV infection and virus entry remain unknown; however, at the cellular level, it is known that expression level and availability of integrins (that permit viral entry), rate of clearance of infected cells and strength of anti-viral type I IFN (interferon) response are key determinants of tissue tropism. Extending findings to improved understanding of transmission requires a standardised approach and adoption of natural routes of infection during experimental study. There has been recognition of the importance of autophagosomes for FMDV entry into the cytoplasm following cell surface receptor binding, and that distinct internal cellular membranes are exploited for viral replication and immune evasion. New roles for viral proteins in blocking type I IFN production and downstream signalling have been identified facilitating research in anti-viral therapeutics. The authors pointed out that more knowledge is available about how infection affects cell protein expression, and research into molecular determinants of capsid stability has aided the development of stable vaccines. Knowledge of viral and host molecular determinates of virulence and infectiousness, and of how phylogenetics may be used to estimate vaccine match and strain distribution, expanded as well. The authors concluded that, with ongoing advances, these areas could translate into significantly improved disease control.

Systemic antibodies administered by passive immunization prevent generalization of the infection by foot-and-mouth disease virus in cattle after oronasal challenge (Barrionuevo et al., 2018[38])

This study investigated the use of systemic antibodies administered by passive immunisation to prevent generalisation of FMD infection in cattle after oro-nasal challenge. Using vaccine-induced immune serum preparations obtained at 7 and 26 days post-vaccination (dpv), the authors showed that circulating antibodies were sufficient to prevent disease generalisation after oro-nasal infection in animals passively transferred with 26-dpv serum but not with the 7-dpv serum. Conversely, conventional FMD vaccination provided clinical protection at 7 dpv, promoting fast and robust antibody responses upon challenge, even though antibody titres were similar to those found in animals passively immunised with 7-dpv serum. These results demonstrate that presence of antigen-specific antibodies is critical to prevent the dissemination of the virus within the animal. Conventional FMD vaccination additionally promoted the deployment of rapid, high titre and isotype-switched antibody responses at systemic and mucosal levels after infection, thus conferring protection even in the presence of low pre-challenge antibody titres.

The need for improved vaccines against foot-and-mouth disease (de Los Santos et al., 2018[39])

While the use of FMD inactivated virus vaccine has played a key role in disease control and eradication

37 Robinson, L., Knight-Jones, T. J. D., Charleston, B., Rodriguez, L. L., Gay, C. G., Sumption, K. J., & Vosloo, W. (2016). Global Foot-and-Mouth Disease Research Update and Gap Analysis: 7–Pathogenesis and Molecular Biology. *Transboundary and Emerging Diseases*, 63(S1), 63-71.

38 Barrionuevo, F., Di Giacomo, S., Bucafusco, D., Ayude, A., Schammas, J., Miraglia, M. C., ... & Perez-Filgueira, M. (2018). Systemic antibodies administered by passive immunization prevent generalization of the infection by foot-and-mouth disease virus in cattle after oronasal challenge. *Virology*, *518*, 143-151.

39 de los Santos, T., Diaz-San Segundo, F., & Rodriguez, L. L. (2018). The need for improved vaccines against foot-and-mouth disease. *Current Opinion in Virology*, *29*, 16-25.

in certain regions of the world, several factors (e.g. rapidly changing environment, increased trade, population growth, international travel and migration) are contributing to disease resurgence, challenging the capabilities of any available vaccine. This article, published in Current opinion in virology, reviews the current knowledge on FMD vaccines, providing an outlook of novel technologies as possible improved alternatives for disease control and eradication.

Rapid detection of foot-and-mouth disease virus using reverse transcription recombinase polymerase amplification combined with a lateral flow dipstick (Wang et al., 2018[40])

An article, published in the Journal of Virological Methods, describes the development of a two-step reverse transcription recombinase polymerase amplification assay combined with lateral flow detection (RPA-LFD) to detect FMD virus (FMDv). With incubation at 38 °C, the authors amplified a region of the 2B gene on the FMDV genome within 20 minutes, using specific primers and a probe. The amplified RPA product can be visualised on a lateral flow dipstick. The RPA-LFD assay was highly sensitive, detecting down to 10 copies of plasmid DNA. The test showed no cross-reactivity with other pathogens causing vesicular lesions. In addition, 143 clinical samples were used to compare RPA-LFD with real-time PCR, with 98.6% concordance between the assays. Therefore, the authors concluded that this RPA-LFD assay would provide a rapid, simple, highly promising approach to be used as point-of-care diagnostics in the field.

40 Wang, H. M., Zhao, G. M., Hou, P. L., Yu, L., He, C. Q., & He, H. B. (2018). Rapid detection of foot-and-mouth disease virus using reverse transcription recombinase polymerase amplification combined with a lateral flow dipstick. *Journal of Virological Methods*, *261*, 46-50.

Transcript Profiling Identifies Early Response Genes against FMDV Infection in PK-15 Cells (Zhang et al., 2018[41])

The mechanism of host's early responses against FMD virus (FMDv) infection is still unclear. In this article, published in Viruses, the authors used a pig kidney cell line (PK-15) as a cell model to investigate the mechanism of early responses to FMDV infection in pig. Four non-treated control and four FMDV-treated PK-15 cells were sequenced with RNA-seq technology, and the differentially expressed genes (DEGs) were analysed. The results showed that 1212 DEGs were expressed in the FMDV-infected PK-15 cells, including 914 up-regulated and 298 down-regulated genes. Kyoto Encyclopedia of Genes and Genome (KEGG) pathways were significantly enriched in the tumour necrosis factor (TNF), cytokine-cytokine receptor interaction, NOD-like receptor, toll-like receptor, NF-κB, and the chemokine signalling pathways. To verify the results of the DEGs, 30 immune-related DEGs (19 up-regulated and 11 down-regulated) were selected for Quantitative Reverse Transcriptase polymerase chain reaction (RT-qPCR) verification. The results showed that RT-qPCR-measured genes exhibited a similar pattern as the RNA-seq analyses. Based on bioinformatics analysis, during FMDV early infection, the authors found that a series of cytokines, such as interleukins (IL6), chemokines (CXCL2, CCL20 and CCL4), and transcription factors (ZFP36, FOS, NFKBIA, ZBTB3, ZNF503, ZNF283, dymeclin (DYM), and orthodenticle homeobox 1 (OTX1)) were involved in the battle between FMDV and the host. This study would provide an additional panel of candidate genes for deciphering the mechanisms of a host's early response against FMDV infection.

Transmission of foot-and-mouth disease from persistently infected carrier cattle to naive cattle via transfer of oropharyngeal fluid (Arzt et al., 2018[42])

An article, published in mSphere, investigates the transmission of foot-and-mouth disease (FMD) from persistently infected carrier cattle to naive cattle via transfer of oropharyngeal fluid. It is often claimed that control and eradication of FMD are impeded by the existence of a persistent, subclinical phase of infection in ruminants (i.e. carriers). However, the epidemiological significance of these FMD virus (FMDV) carriers is uncertain. In this paper, the contagion associated with FMDV carrier cattle was investigated by exposure of susceptible cattle and pigs to oropharyngeal fluid (OPF) samples or tissues harvested from persistently infected cattle. Naive cattle were inoculated through intranasopharyngeal deposition of unprocessed OPF samples that had been collected from FMDV carriers at 30 days post infection. These inoculated cattle developed clinical FMD, and the severity of disease they developed was similar to that of animals that had been infected with a high-titre inoculum. In contrast, pigs exposed via intraoropharyngeal inoculation of the same OPF samples or via ingestion of nasopharyngeal tissues harvested from the same cohort of persistently infected cattle did not develop FMD. These findings indicated that there was a demonstrable contagion associated with FMDV carrier cattle despite the lack of evidence for transmission by direct contact. The findings of this work provided novel information that should be considered for FMD risk mitigation strategies. IMPORTANCE Foot-and-mouth disease (FMD) is a viral disease of livestock with substantial impact on agricultural production and subsistence farming on a global scale. Control of FMD is impeded by the existence of a prolonged asymptomatic carrier phase during which infected cattle shed low quantities of infectious virus in oropharyngeal fluid (OPF) for months to years after infection. The epidemiological significance of FMD virus (FMDV) carriers is unresolved. However, the existence of the FMDV carrier state has substantial impact on international trade in animal products. The study demonstrated that transfer of OPF from persistently infected FMDV carrier cattle to naive cattle led to fulminant clinical FMD. It was thus demonstrated that, although the risk for disease transmission under natural conditions is considered to be low, there is detectable contagion associated with FMDV carrier cattle. This finding is important for optimisation of FMD risk mitigation strategies.

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41 Zhang, T., Chen, H., Qi, L., Zhang, J., Wu, R., Zhang, Y., & Sun, Y. (2018). Transcript Profiling Identifies Early Response Genes against FMDV Infection in PK-15 Cells. *Viruses*, *10*(7), 364.

42 Arzt, J., Belsham, G. J., Lohse, L., Bøtner, A., & Stenfeldt, C. (2018). Transmission of foot-and-mouth disease from persistently infected carrier cattle to naive cattle via transfer of oropharyngeal fluid. *Msphere*, *3*(5), e00365-18.

Versatility of the adenovirus-vectored foot-and-mouth disease vaccine platform across multiple foot-and-mouth disease virus serotypes and topotypes using a vaccine dose representative of the AdtA24 conditionally licensed vaccine (Barrera et al., 2018[43])

This study investigated the serotype- and topotype versatility of a replication-deficient human adenovirus serotype 5 vectored foot-and-mouth disease (FMD) vaccine platform (AdtFMD). Sixteen AdtFMD recombinant subunit monovalent vaccines targeting twelve distinct FMD virus (FMDV) serotype/topotypes in FMD Regional Pools I-VII were constructed. The AdtA24 serotype conditionally licensed vaccine served as the basis for vaccine design and target dose for cattle clinical trials. Several vaccines contained an additional RGD motif genetic insertion in the adenovector fiber knob, and/or a full-length 2B gene insertion in the FMDV P1 gene cassette. In 13 of the 22 efficacy studies conducted, naïve control and AdtFMD vaccinated cattle were challenged intradermolingually at 2 weeks post-vaccination using a FMDV strain homologous to the AdtFMD vaccine strain. Each of the 16 AdtFMD vaccines were immunogenic based on the presence of homologous neutralising antibodies in the serum of approximately 90% of total vaccinates (n=375) on the day of challenge. Importantly, for 75% of vaccines tested, the effective dose that conferred 100% protection against clinical FMD was identical to or in some cases lower than, the minimum protective dose for the conditionally licensed AdtA24 vaccine formulated with ENABL® adjuvant. Results also confirmed the capability of the AdtFMD vaccine platform to allow differentiation of infected from vaccinated animals (DIVA) across the five FMDV serotypes evaluated. Collectively, this comprehensive set of FMD cattle vaccine dose ranging studies highlighted the serotype- and topotype versatility of the AdtFMD vaccine platform for further development, licensure, and application in FMD outbreak control and disease eradication efforts.

The different tactics of foot-and-mouth disease virus to evade innate immunity (Medina et al., 2018[44])

An article, published in Frontiers in Microbiology, reviews different tactics of foot-and-mouth disease virus (FMDV) to evade innate immunity, discussing FMDV virulence factors and the host immune footprint that characterise infection in cell culture and in the natural hosts. FMDV elicits an immune response mainly mediated by type I and type III IFNs. To overcome the strong antiviral response induced by these cytokines, FMDV has evolved many strategies exploiting each region of its small RNA genome. These include: (a) inhibition of IFN induction at the transcriptional and translational level, (b) inhibition of protein trafficking; (c) blockage of specific post-translational modifications in proteins that regulate innate immune signalling; (d) modulation of autophagy; (e) inhibition of stress granule formation; and (f) in vivo modulation of immune cell function.

Safe and cost-effective protocol for shipment of samples from foot-and-mouth disease suspected cases for laboratory diagnostic (Romey et al., 2018[45])

This article, published in Transboundary and Emerging Diseases, describe the development of a safe and cost-effective protocol for shipment of samples from foot-and-mouth disease (FMD) suspected cases for laboratory diagnosis. The identification of circulating virus strains in endemic regions is fundamental to implement adequate outbreak control measures. However, due to the high biological risk and the

43 Barrera, J., Brake, D. A., Schutta, C., Ettyreddy, D., Kamicker, B. J., Rasmussen, M. V., ... & Brough, D. E. (2018). Versatility of the adenovirus-vectored foot-and-mouth disease vaccine platform across multiple foot-and-mouth disease virus serotypes and topotypes using a vaccine dose representative of the AdtA24 conditionally licensed vaccine. Vaccine, 36(48), 7345-7352.

44 Medina, G. N., Segundo, F. D. S., Stenfeldt, C., Arzt, J., & de los Santos, T. (2018). The different tactics of foot-and-mouth disease virus to evade innate immunity. Frontiers in Microbiology, 9, 2644.

45 Romey, A., Relmy, A., Gorna, K., Laloy, E., Zientara, S., Blaise-Boisseau, S., & Bakkali Kassimi, L. (2018). Safe and cost-effective protocol for shipment of samples from Foot-and-Mouth Disease suspected cases for laboratory diagnostic. Transboundary and emerging diseases, 65(1), 197-204.

requirement for biological samples to be shipped frozen, the cost of shipping samples becomes one of major obstacles hindering submission of suspected samples to reference laboratories for virus identification. The protocol developed by the authors is based on the inactivation of FMD virus (FMDV) on lateral flow device (LFD, penside test routinely used in the field for rapid immunodetection of FMDV), allowing its subsequent detection and typing by RT-PCR and recovery of live virus upon RNA transfection into permissive cells. After live FMDV collection onto LFD strip and soaking in 0.2% citric acid solution, the virus is totally inactivated. Viral RNA is still detectable by real-time RT-PCR following inactivation, and the virus strain can be characterised by sequencing of the VP1 coding region. In addition, live virus can be rescued by transfecting RNA extract from treated LFD into cells. In the view of the authors, this protocol should help promote submission of FMD suspected samples to reference laboratories (by reducing the cost of sample shipping) and thus characterisation of FMDV strains circulating in endemic regions.

Quantitative impacts of incubation phase transmission of foot-and-mouth disease virus (Arzt et al., 2019[46])

An article, published in Scientific Report, estimated the occurrence of transmission of foot-and-mouth disease (FMD) during the incubation phase amongst group-housed pigs, applying a Bayesian modelling approach. The primary outcome was that transmission occurred approximately one day prior to development of visible signs of disease (posterior median 21 hours, 95% CI: 1.1–45.0). Updated disease state durations were incorporated into a simulation model to examine the importance of addressing preclinical transmission in the face of robust response measures. Simulation of FMD outbreaks in the USA pig production sector demonstrated that including a preclinical infectious period of one day would result in a 40% increase in the median number of farms affected (166 additional farms and 664,912 pigs euthanised) compared to the scenario of no preclinical transmission, assuming suboptimal outbreak response. These findings emphasise the importance of considering transmission of FMD during the incubation phase in modelling and response planning.

The evolution of a super-swarm of foot-and-mouth disease virus in cattle (Arzt et al., 2019[47])

The extensive viral population diversity and rapid, continuous mutation of circulating FMD viruses (FMDVs) pose significant obstacles to the control and ultimate eradication of this important transboundary pathogen. A study, published in PLoS ONE, investigated the mechanisms contributing to within-host evolution of FMDV in a natural host species (cattle). Specifically, vaccinated and non-vaccinated cattle were infected with FMDV under controlled, experimental conditions and subsequently sampled for up to 35 days to monitor viral genomic changes as related to phases of disease and experimental cohorts. Consensus-level genomic changes across the entire FMDV coding region were characterised through three previously defined stages of infection: early, transitional, and persistent. The authors concluded that viral evolution occurred via a combination of two mechanisms: emergence of full-genomic minority haplotypes from within the inoculum super-swarm, and concurrent continuous point mutations. Phylogenetic analysis indicated that individuals were infected with multiple distinct haplogroups that were pre-existent within the ancestral inoculum used to infect all animals. Multiple shifts of dominant viral haplotype took place during the early and transitional phases of infection, whereas few shifts occurred during persistent infection. Overall, this work suggests that the establishment of the carrier state is not associated with specific viral genomic characteristics. In the authors views, these insights into FMDV population dynamics would have important implications for virus sampling methodology and molecular epidemiology.

46 Arzt, J., Branan, M. A., Delgado, A. H., Yadav, S., Moreno-Torres, K. I., Tildesley, M. J., & Stenfeldt, C. (2019). Quantitative impacts of incubation phase transmission of foot-and-mouth disease virus. *Scientific reports*, 9(1), 2707.

47 Arzt, J., Fish, I., Pauszek, S. J., Johnson, S. L., Chain, P. S., Rai, D. K., ... & Stenfeldt, C. (2019). The evolution of a super-swarm of foot-and-mouth disease virus in cattle. PloS one, 14(4), e0210847.

5. Helminths

Global network: Livestock Helminth Research Alliance (LiHRA)

The Livestock Helminth Research Alliance (LiHRA) was founded in December 2014, comprising 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 16 member organisations from 10 European countries. Currently, efforts are underway to invite members from other continents. 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. Contributions in recent years include: update of DISCONTOOLS information for nematodes and liver fluke; and response to 6 international grant calls (2 Horizon 2020 – Programme Food security and sustainable agriculture, 2 Horizon 2020 Marie-Sklodowska-Curie Action,1 COST Action, and 1 joint call FACCE ERA-GAS, ERA-NET Susan, ICT Agri). Of these the COST Action COMBAR was successful and runs until September 2021. LiHRA-members wrote a number of peer reviewed articles covering the gaps in research on the control of gastrointestinal nematodes and liver fluke in livestock as well as a vision paper for the control of helminth infections in ruminants by 2030. LiHRA members contributed to the development of the STAR-IDAZ helminth research road maps for diagnostics, vaccines, therapeutics and control.

DISCONTOOLS

The database was last updated in 2018 for liver fluke and 2015 for nematodes. Gaps identified in the field of diagnostics, vaccines and pharmaceuticals are presented below. Other knowledge gaps and more information are available at www.discontools.eu.

Nematodes

Diagnostics

In ruminants, coprological (microscopic) methods are used for all gastrointestinal (GI) nematodes of all hosts to identify and quantify eggs and with coproculture to identify L3 stage larvae. Serological methods involve measuring serum pepsinogen levels to assess the degree of damage/extent of exposure to abomasal nematode infections. Antibody levels against crude extract of Ostertagia ostertagi in bulk-tank milk or serum are used to assess nematode exposure in adult cows. Morbidity markers have been described in sheep. Pig nematodes are mainly diagnosed by faecal examination for eggs and occasional reports from abattoir of milk spots in the liver, only indicative of recent Ascaris suum exposure. Observing the worms in faeces reassures the farmer of the need to treat the animal.

The conventional diagnosis of nematode infections is laborious and expensive, and often not

informative in providing a decision on whether or not to treat. A key problem is to identify those animals requiring treatment in order to avoid unnecessary use of anthelmintics. Non-invasive and automated sampling methods and assays (e.g. milk, meat-juice, body condition scoring) are required. Further development of existing tests to make them suitable for high-throughput platforms and the development of pen-side tests for user friendly (low input) on-farm monitoring and rapid detection of parasitic infections would be beneficial. Other requirements include novel tests for the early detection of anthelmintic resistance and the interpretation of results, identification of the specific proteins or sequences for species differentiation and the novel genetic markers associated with host resistance/ resilience.

Vaccines

Prototype vaccines against Haemonchus contortus reduce worm numbers and worm egg output by > 90%. Prototype vaccines against Ostertagia ostertagi reduce worm egg output by 60% during a two-month challenge period. The main shortcomings include a lack of cross-protection against other important nematodes and possible need for repeated administrations. The required efficacy has been defined for some species by experimental infection and/or by modelling but there is a requirement to define efficacy in the field, probably at the level required to reduce or eliminate the economic impact of the disease. Vaccines for all of the important gastrointestinal nematodes might be commercially viable as monovalent vaccines. However, the ambition should be to develop polyvalent vaccines that provide protection against all relevant gastrointestinal nematode species in a single vaccine. Effective recombinant vaccines to allow mass production are required.

Pharmaceuticals

Control of GI nematodes relies largely on anthelmintics. All anthelmintics used in livestock are very effective at reducing susceptible worm burdens. Possible drawbacks to the use of anthelmintics may include: (a) the increasing incidence of anthelmintic resistance (AR); (b) the reduced development of natural immunity against nematodes; and (c) consumer concerns (often not justified) regarding drug residues in food products and in the environment. Instead of blanket treatments, future treatment strategies could benefit from selective treatment of only those animals requiring treatment. This means of optimising anthelmintic usage to both control nematodes and maintain efficacy.

The dependence on anthelmintics is not without risk as the spread of anthelmintic resistance (AR) is an emerging problem. The prevalence of AR varies geographically, depending on the livestock species involved and the drugs used. Benzimidazole-resistant and Macrocyclic lactones-resistant nematodes are widely reported in sheep/goats of several temperate European countries. Resistance to levamisole is present in sheep and goat parasites, though at a lower level. In cattle AR has been reported, however, until now it is mainly limited to Macrocyclic lactones resistance of Cooperia spp. In pigs AR has been demonstrated for Oesophagostomum spp. in Denmark and Germany (pyrantel, levamisole, benzimidazoles), and may be an overlooked problem.

Liver fluke

Diagnostics

A range of diagnostic tools are available to detect infection, but few are used to detect disease. There are commercial antibody detection tests for cattle but not for sheep, but these detect evidence of exposure not necessarily current infection. Little information is available about how quickly antibody levels decline in response to treatment or loss of infection. Bulk tank tests are available for dairy cattle to enable herd-level estimates of exposure. There is a need for i) pen-side tests, ii) herd level tests to identify heavily infected beef herds, iii) tests for diagnosis especially for acute infection in sheep or pre-patent infections in any host and iv) for the rapid diagnosis of recent infections before seroconversion.

Also, copro-antigen detection ELISAs, that can be used in sheep and cattle, are available commercially. The modified version requires further evaluation, and this test does not appear to work in horses. Faecal egg counts remain the gold standard to confirm live infection but fail to diagnose infection in the high-risk pre-patent period thereby delaying appropriate management responses. Faecal egg count kits are available but whilst faecal egg counts are a useful indicator of infection these need validation for composite samples in cattle. Rumen flukes (paramphistomes) are becoming an increasing problem in

some countries but diagnostics to differentiate between rumen fluke and liver fluke are not available.

Vaccines

There are no vaccines currently available, but a number are under development. Research is required into how efficacious vaccines should be in order to have an effect in the field either on reducing transmission or generating sufficient immunity to protect the individual against the disease. Integration of infection transmission reduction and disease incidence into a single protective score would be valuable in assessing the threshold for commercial viability. DIVA based vaccines, and corresponding diagnostic tests, are also important to reduce unnecessary anthelmintic treatment.

Pharmaceuticals

The prophylactic use of anthelmintics is currently the main method for prevention and control. There are a number of anthelmintics available, triclabendazole being the anthelmintic of choice because of its proven efficacy against young immature stages of Fasciola spp. Other than triclabendazole (TCBZ) there are no fully effective drugs against young juvenile stages of the parasite which are highly pathogenic.

Resistance in parasite populations to TCBZ has been reported in many countries (Australia, Europe and S. America) however a concerted effort to track the emergence and prevalence of resistance to TCBZ is needed. Integrated research into strategic treatment regimes, and subsequent re-infection diagnosis, reducing reliance on anthelmintics is required. The mode of action of current flukicides is not well understood and more work is required to investigate their modes of action and the mechanisms of drug resistance.

Recent developments

Modelling the consequences of targeted selective treatment strategies on performance and emergence of anthelmintic resistance amongst grazing calves (Berk et al., 2016[48])

The development of anthelmintic resistance by helminths can be slowed by maintaining refugia on pasture or in untreated hosts. Targeted selective treatments (TST) may achieve this through the treatment only of individuals that would benefit most from anthelmintic, according to certain criteria. However, TST consequences on cattle are uncertain, mainly due to difficulties of comparison between alternative strategies. In a paper published in International Journal for Parasitology – Drugs & Drug Resistance, Berk et al. developed a mathematical model to compare: 1) the most 'beneficial' indicator for treatment selection and 2) the method of selection of calves exposed to Ostertagia ostertagi, i.e. treating a fixed percentage of the population with the lowest (or highest) indicator values versus treating individuals who exceed (or are below) a given indicator threshold. The indicators evaluated were average daily gain (ADG), faecal egg counts (FEC), plasma pepsinogen, combined FEC and plasma pepsinogen, versus random selection of individuals. Treatment success was assessed in terms of benefit per R (BPR), the ratio of average benefit in weight gain to change in frequency of resistance alleles R (relative to an untreated population). The optimal indicator in terms of BPR for fixed percentages of calves treated was plasma pepsinogen. When calves were treated according to threshold criteria, ADG was the best target indicator for treatment. This was also the most beneficial strategy overall, with a significantly higher BPR value than any other strategy, but its degree of success depended on the chosen threshold of the indicator. The study showed strong support for TST, with all strategies showing improvements on calves treated selectively, compared with whole-herd treatment at 3, 8, 13 weeks post-turnout. The developed model appeared capable of assessing the consequences of other TST strategies on calf populations.

48 Berk, Z., Laurenson, Y. C. S. M., Forbes, A. B., & Kyriazakis, I. (2016). Modelling the consequences of targeted selective treatment strategies on performance and emergence of anthelmintic resistance amongst grazing calves. *International Journal for Parasitology-Drugs and Drug Resistance, 6*(3), 258-271.

Climate-driven longitudinal trends in pasture-borne helminth infections of dairy cattle (Charlier et al., 2016[49])

There is a growing concern that climate change increases helminth disease frequency and intensity. In Europe, these concerns stem from case reports and theoretical life cycle models assessing the effects of climate change scenarios on helminth epidemiology. In an article published in International Journal for Parasitology, Charlier et al. reported the first study on climate-driven trends in helminth infections of cattle based on a cohort of randomly selected farms. One thousand, six hundred and eighty dairy farms were monitored over an 8-year period for the two major helminth infections in a temperate climate region based on bulk-tank milk samples and climate-driven trends were investigated by multivariable linear mixed models. The general levels of exposure to Fasciola hepatica decreased over the study period while those to Ostertagia ostertagi increased, and this could at least be partially explained by meteorological factors. The longitudinal trends varied according to the altitude and the agricultural region of the farm. The authors concluded that longitudinal epidemiological data from sentinel farms combined with meteorological datasets are key to understand the effects of climate on infectious disease dynamics and recommended to set up longitudinal monitoring programmes of helminth infections across Europe to promote animal health and productivity.

Progress in the development of subunit vaccines for gastrointestinal nematodes of ruminants (Matthews et al., 2016[50])

In a paper published in Parasite Immunology, Matthews et al. reviewed the current status of subunit vaccine development for a number of important gastrointestinal nematodes of cattle and sheep, with a focus on the limitations and problems encountered so far, and suggestions as to how these hurdles might be overcome. The authors argued that subunit vaccines would probably be the only valid option for the long-term control of ruminant parasitic nematodes given the increasing ubiquity of multidrug resistance in a range of worm species across the world. The development of a subunit vaccine against multicellular parasites to the point of practical application would be a ground-breaking step in the control of these important endemic infections of livestock.

Utilization of composite fecal samples for detection of anthelmintic resistance in gastrointestinal nematodes of cattle (George et al., 2017[51])

Presently, the faecal egg count reduction test (FECRT) is the only means available for detection of resistance to anthelmintics in sheep or cattle herds at the farm level. However, the FECRT is labour and cost intensive, and consequently is only rarely performed on sheep or cattle farms unless for research purposes. In a paper published in Veterinary Parasitology, George et al. proposed and evaluated the use of composite samples as a practical and more cost-effective tool to assess anthelmintic resistance. They reported excellent agreement in mean faecal egg count and faecal egg count reduction of individual and composite samples.

Genetic line comparisons and genetic parameters for endoparasite infections and test-day milk production traits (May et al., 2017[52])

In a paper published in Journal of Dairy Science, May et al. evaluated the potential of genetic selection to improve dairy cow resistance against endoparasite infections. They (1) compared different Black and

49 Charlier, J., Ghebretinsae, A. H., Levecke, B., Ducheyne, E., Claerebout, E., & Vercruysse, J. (2016). Climate-driven longitudinal trends in pasture-borne helminth infections of dairy cattle. *International Journal for Parasitology, 46*(13-14), 881-888.

50 Matthews, J. B., Geldhof, P., Tzelos, T., & Claerebout, E. (2016). Progress in the development of subunit vaccines for gastrointestinal nematodes of ruminants. *Parasite Immunology, 38*(12), 744-753.

51 George, M. M., Paras, K. L., Howell, S. B., & Kaplan, R. M. (2017). Utilization of composite fecal samples for detection of anthelmintic resistance in gastrointestinal nematodes of cattle. *Veterinary Parasitology, 240*, 24-29.

52 May, K., Brugemann, K., Yin, T., Scheper, C., Strube, C., & Konig, S. (2017). Genetic line comparisons and genetic parameters for endoparasite infections and test-day milk production traits. *Journal of Dairy Science, 100*(9), 7330-7344

White dairy cattle selection lines for endoparasite infections and (2) estimated the genetic (co)variance components for endoparasite and test-day milk production traits within the Black and White cattle population. A total of 2,006 faecal samples were taken during 2 farm visits in summer and autumn 2015 from 1,166 cows kept in 17 small- and medium-scale organic and conventional German grassland farms. Faecal egg counts were determined for gastrointestinal nematodes (FEC-GIN) and flukes (FEC-FLU), and faecal larvae counts for the bovine lungworm Dictyocaulus viviparous (FLC-DV). The lowest values for gastrointestinal nematode infections were identified for genetic lines adopted to pasture-based production systems, especially selection lines from New Zealand. Heritabilities were low for FEC-GIN and FLC-DV, but moderate for FEC-FLU. Genetic correlations were negative between FEC-GIN and milk yield (MY) until DIM 85, and between FEC-FLU and MY until DIM 215. Genetic correlations between FLC-DV and MY were negative throughout lactation, indicating improved disease resistance for high-productivity cows. Genetic correlations between FEC-GIN and somatic cell score were positive, indicating similar genetic mechanisms for susceptibility to udder and endoparasite infections. The authors concluded that the moderate heritability for FEC-FLU suggest inclusion of FEC-FLU into overall organic dairy cattle breeding goals to achieve long-term selection response for disease resistance.

Multiplexed-tandem PCR for the specific diagnosis of gastrointestinal nematode infections in sheep: an European validation study (Roeber et al., 2017[53])

In a paper published in Parasites & Vectors, Roeber et al. reported the development and validation of an automated multiplexed-tandem PCR for the diagnosis and identification of patent infections with key genera of gastrointestinal nematodes of sheep. The authors concluded that the MT-PCR platform was an advanced method for the species/genus-specific diagnosis of gastrointestinal nematode infections in small ruminants and had demonstrated utility when deployed in different countries and climatic zones. The platform is user-friendly due to the largely automated procedure and has high versatility as it can achieve a specific diagnosis from different types of sample templates, including larval culture and faecal samples. With appropriate modifications of the primers used, the MT-PCR platform also provides potential for the diagnosis of a variety of other pathogens of veterinary or medical importance.

Strategies to optimize the efficacy of anthelmintic drugs in ruminants (Lanusse et al., 2018[54])

Anthelmintic resistance in human and animal pathogenic helminths has been spreading in prevalence and severity. In this paper, the authors discuss the use of available pharmacology-based information for the design of successful future control approaches. The different strategies to optimize anthelmintic therapy in ruminants under the current drug-resistance scenario is described. The authors emphasize the need for further integrated pharmaco-parasitological knowledge to extend the lifespan of both traditional and novel anthelmintic compounds, and to progress in the identification of complementary/alternative measures of parasite control in livestock animals.

Exploring the mechanisms of resistance to Teladorsagia circumcincta infection in sheep through transcriptome analysis of abomasal mucosa and abomasal lymph nodes (Chitneedi et al., 2018[55])

The present study exploited the RNA-seq technology to analyse the transcriptome of target tissues

53 Roeber, F., Morrison, A., Casaert, S., Smith, L., Claerebout, E., & Skuce, P. (2017). Multiplexed-tandem PCR for the specific diagnosis of gastrointestinal nematode infections in sheep: an European validation study. *Parasites & Vectors, 10*.

54 Lanusse, C., Canton, C., Virkel, G., Alvarez, L., Costa-Junior, L., Lifschitz, A . (2018). Strategies to optimize the efficacy of anthelmintic drugs in ruminants. *Trends in Parasitology* 34, 8.

55 Chitneedi, P.K., Suarez-Vega, A., Martinez-Valladares, M., Arranz, J.J., Gutiérrez-Gil, B. (2018). Exploring the mechanisms of resistance to *Teladorsagia circumcincta* infection in sheep through transcriptome analysis of abomasal mucosa and abomasal lymph nodes. *Veterinary Research* 49, 39.

affected by the Teladorsagia circumcincta infection in two groups of adult ewes showing different statuses against gastrointestinal nematode (GIN) infection with the aim of identifying genes linked to GIN infection resistance in sheep. RNA samples were obtained from abomasal mucosa and lymph node tissues and RNA-Seq datasets were generated using an Illumina HiSeq 2000 sequencer. The distribution of the genes based on their expression level were very similar among the two different tissues and conditions. The differential expression analysis only identified common differentially expressed genes (DEGs), a total of 106, in the lymph node samples which were considered as GIN-activated. The enrichment analysis performed for these GIN-activated genes identified some pathways related to cytokine-mediated immune responses and the PPARG signalling pathway as well as markers related to inflammation and gastro-intestinal diseases as enriched. A systematic comparison with the results of previous studies confirmed the involvement of genes such as ITLN2, CLAC1 and galectins, in the immune mechanism activated against T. circumcincta in resistant sheep.

Concurrent treatment with a macrocyclic lactone and benzimidazole provides season long performance advantages in grazing cattle harbouring macrocyclic lactone resistant nematodes (Edmonds et al., 2018[56])

In this study, the authors investigated the efficacy of concurrent treatment at pasture turnout with an injectable macrocyclic lactone and an oral benzimidazole, referred to as "conventional" anthelmintics, and compared it to treatment with conventional macrocyclic lactone alone or an injectable macrocyclic lactone with extended activity of 100 days or longer. During the first 32 days, the concurrent therapy provided nearly 100% efficacy based on faecal egg count reduction and a 19.98% improvement in total weight gain compared to controls (P = 0.039). At the end of the 118-day study and past the approved efficacy for the conventional anthelmintics, the concurrent therapy with conventional anthelmintics provided a 22.98% improvement in total weight gain compared to controls (P = 0.004). The 118-day improvement in weight gain for the extended release macrocyclic lactone group (29.06% compared to control) was not statistically different from the concurrent therapy with conventional anthelmintics. The authors concluded that concurrent treatment with a conventional macrocyclic lactone and benzimidazole may provide production benefits early in the grazing period that continue throughout the entire period for cattle harbouring macrocyclic lactone resistant nematodes. By using two different anthelmintic classes together, macrocyclic lactone resistant parasites were effectively controlled early in the period. Furthermore, the use of an effective conventional anthelmintic treatment regimen without an extended period of drug release may help to promote refugia and decrease the further selection for anthelmintic resistant parasites.

Transcriptomic profiling of nematode parasites surviving vaccine exposure (Sallé et al., 2018[57])

Vaccination against gastrointestinal nematodes is considered to be a sustainable and cost-effective strategy for the future. Currently, Barbervax® for the ruminant strongylid Haemonchus contortus is the only registered subunit vaccine for a nematode parasite. As this vaccine comprises a limited number of proteins, there is potential for selection of nematodes with altered sequences or expression of the vaccine antigens and thus to confer resistance against vaccination. Here, the authors compared the transcriptome of H. contortus populations from sheep vaccinated with Barbervax® with worms from control animals. Barbervax® antigens are native integral membrane proteins isolated from the brush border of the intestinal cells of the adult parasite and many of those are proteases. The findings provide

56 Edmonds, M.D., Vatta, A.F., Marchiondo, A.A., Vanimisetti, H.B., Edmonds, J.D. (2018). Concurrent treatment with a macrocyclic lactone and benzimidazole provides season long performance advantages in grazing cattle harboring macrocyclic lactone resistant nematodes. *Veterinary Parasitology* 252, 157-162.

57 Sallé, G., Laing, R., Cotton, J.A., Maitland, K., Martinelli, A., Holroyd, N., Tracey, A., Berriman, M., Smith, W.D., Newlands, G.F.J., Hanks, E., Devaney, E., Britton, C. (2018). Transcriptomic profiling of nematode parasites surviving vaccine exposure. *International Journal for Parasitology* 48, 395-402.

no evidence for changes in expression of genes encoding Barbervax® antigens in the surviving parasite populations. However, surviving parasites from vaccinated animals showed increased expression of other proteases and regulators of lysosome trafficking, and displayed up-regulated lipid storage and defecation abilities that may have circumvented the effect of the vaccine. Implications for other potential vaccines for human and veterinary nematodes are discussed.

Population genomic and evolutionary modelling analyses reveal a single major QTL for ivermectin drug resistance in the pathogenic nematode, Haemonchus contortus (Doyle et al., 2019[58])

Large scale use of anthelmintics has driven the evolution of resistance in a number of species that infect livestock and companion animals. Understanding the mechanisms by which resistance evolves is the focus of increasing interest; robust genetic analysis of helminths is challenging, and although many candidate genes have been proposed, the genetic basis of resistance remains poorly resolved. In this study, the authors present a genome-wide analysis of two genetic crosses between ivermectin resistant and sensitive isolates of the parasitic nematode Haemonchus contortus, an economically important gastrointestinal parasite of small ruminants and a model for anthelmintic research. Whole genome sequencing of parental populations, and key stages throughout the crosses, identified extensive genomic diversity that differentiates populations, but after backcrossing and selection, a single genomic quantitative trait locus (QTL) localised on chromosome V was revealed to be associated with ivermectin resistance. This QTL was common between the two geographically and genetically divergent resistant populations and did not include any leading candidate genes, suggestive of a previously uncharacterised mechanism and/or driver of resistance. Despite limited resolution due to low recombination in this region, population genetic analyses and novel evolutionary models supported strong selection at this QTL, driven by at least partial dominance of the resistant allele, and that large resistance-associated haplotype blocks were enriched in response to selection. The authors thus have described the genetic architecture and mode of ivermectin selection, revealing a major genomic locus associated with ivermectin resistance, the most conclusive evidence to date in any parasitic nematode. This study highlighted a novel genome-wide approach to the analysis of a genetic cross in non-model organisms with extreme genetic diversity, and the importance of a high-quality reference genome in interpreting the signals of selection so identified.

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, conducted a 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 site 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 is working on the development of a roadmap for Epidemiology and Control Strategies for PRRS.

58 Doyle, S.R., Illingworth C.J.R., Laing, R., Bartley, D.J., Redman, E., Martinelli, A., Holroyd, N., Morrison, A.A., Rezansoff, A., Tracey, A;, Devanay, E., Berriman, M., Sargison, N., Cotton, J.A., Gilleard, J.S. (2019). Population genomic and evolutionary modelling analyses reveal a single major QTL for ivermectin drug resistance in the pathogenic nematode, Haemonchus contortus *BMC Genomics* 20:218.

DISCONTOOLS

The updated information on PRRS was published in June 2019. Below follows an extraction of identified gaps in the field of diagnostics, vaccines and pharmaceuticals. Other knowledge gaps and more information are available at www.discontools.eu.

Diagnostics

A number of serological methods are available for diagnosis. In general, they have good specificity and sensitivity. For initial monitoring/diagnosis, a wide variety of ELISAs has been developed, capable of identifying antibodies induced by strains of both PRRSV-1 and PRRS-2. In order to confirm the initial positive results, and to differentiate genotypes/strains, ORF5 ELISA and the indirect immunoperoxidase monolayer assay (IPMA) or indirect immunofluorescence (IIF) using alveolar macrophages and MARC-145 cells can be applied. Diagnostic kits (PCRs, ELISAs) are available worldwide and are very effective in determining the presence of PRRS virus in a population. Nevertheless, it is questionable if they can pick up all circulating isolates considering the ability of the PRRS virus to mutate rapidly. PCRs/ELISAs should be continuously validated 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 database should be created that allows simultaneous comparison of PRRS isolates in neighbouring countries. This requires regular pathological studies for new virus strains, isolation of the virus, whole genome sequencing and antisera production.

Vaccines

Both live attenuated and inactivated vaccines are available containing either PRRSV-1 or PRRSV-2. Inactivated vaccines are safe but not efficacious, as it has been demonstrated that they cannot control viremia post-challenge by themselves. They can only boost the existing immune response in sows. Inactivated vaccines do not protect naïve animals and give only boost reactions when the animals have been previously exposed to the field virus or Modified Live vaccines (MLV). MLVs have been proven to reduce clinical signs, viremia and shedding post-challenge, as well as to reduce virus transmission in vaccinated populations. In regards to safety, some limited horizontal and vertical spread can be found, as well as vaccine strain shedding. These characteristics differ between vaccine strains. Although initially it was assumed that the efficacy of attenuated vaccines depends on the homology to the field virus, it has been proven recently that the vaccine efficacy also depends on the capacity of the vaccine to induce cellular immunity and, probably, virus-neutralising antibodies. Nevertheless, vaccine efficacy is always partial. Therefore, proper vaccination regimes should be applied together with strict biosecurity measures.

As vaccine protection is partial, there is an urgent need for new generation vaccines that could provide universal protection. Additionally, it would be important to be able to differentiate vaccinated animals from infected ones. To achieve this development, new approaches of vaccine production, such as multivalent vaccines or subunit vaccines should be considered.

Pharmaceuticals

No antivirals are available against the PRRS virus, but high levels of antibiotics are used against the bacterial co-infections.

Recent developments

Antiviral Strategies against PRRSV Infection (Du et al., 2017[59])

An article published on Trends in Microbiology discussed the limited knowledge that is available on the virology, origin, and evolution of PRRSV and the host's immune response, and its impediment to

59 Du, T., Nan, Y., Xiao, S., Zhao, Q., & Zhou, E. M. (2017). Antiviral Strategies against PRRSV Infection. *Trends in Microbiology*, 25(12), 968-979.

develop effective method for diseases eradication. The authors reviewed recent advances in anti-PRRSV research, especially focusing on those techniques with the potential to transform current anti-PRRSV strategies. Gene-editing of CD163 might represent a potential way to develop PRRSV resistant pigs, and the identification of both anti- or pro-PRRSV miRNAs offers alternative targets for gene editing, but both those approaches would face the ethical of acceptance of GMOs (genetically modified organisms) as food sources in most countries. The review then summarises some antiviral strategies against PRRSV infection that could be useful to block semen-mediated PRRSV transmission and persistent infection of piglets that survived challenge in utero. Since pigs are the only host for this virus, global campaigns using large-scale immunisation with effective vaccines may quickly eradicate this pathogen worldwide. The authors listed two novel strategies that hold great promise for the development of such a vaccine. One strategy involves artificially swapping or shuffling genetic elements to create cross-protective chimeric virus vaccines. The second entails the use of IFN-inducible strains to restore the host immune response. Both strategies show promise when compared with conventional MLVs based on single strains. Lastly, the authors recognised that inactivated PRRSV vaccine would be promising as a therapeutic or antiviral agent for PRRSV-positive herds.

ORF1a of highly pathogenic PRRS attenuated vaccine virus plays a key role in neutralizing antibody induction in piglets and virus neutralization in vitro (Leng et al., 2017[60])

An article published in the Virology Journal investigated the effect of exchanging three coding DNA sequence with untranslated regions (UTR) in six PRRS chimeric viruses (using infectious clones of two PRRSV attenuated live vaccine strains, HuN4-F112 and CH-1R), on the production of PRRS neutralising antibodies (NA) in vivo and in vitro. All three fragments (5' UTR + open reading frame (ORF)1a, ORF1b, and ORF2–7 + 3'UTR) could affect the replication efficiencies of rHuN4-F112 and rCH-1R in vitro. Additionally, both 5'UTR + ORF1a and ORF2–7 + 3'UTR affected the anti-N antibody and NA responses targeting rHuN4-F112 and rCH-1R in piglets. The 5'UTR + ORF1a region of HuN4-F112 played a key role in inducing NAs in piglets. Furthermore, the authors confirmed for the first time that ORF1a contains a neutralisation region. This study provided important information that can be used for further study of the generation of anti-PRRSV NAs.

Strategies to broaden the cross-protective efficacy of vaccines against porcine reproductive and respiratory syndrome virus (Vu et al., 2017[61])

An article published in Veterinary Microbiology investigated the limit of commercially available PRRS vaccines, summarising the impediments for the development of a highly protective PRRS vaccine and reviewing the vaccinology approaches that have been attempted to overcome the substantial genetic variation among PRRSV isolates. For each of different methods that can be used to expand the antigenic coverage of PRRS (i.e. multi-strain vaccine, chimeric virus, DNA shuffling and centralised immunogen), the authors described recent advances and future perspectives. The approaches that rely on the use of molecular techniques to manipulate the viral genome, such as DNA shuffling and centralised antigens, appeared to be the most worth mentioning. The data from the immunisation/challenge experiments conducted with the synthetic PRRSV-CON (consensus genome) strain provide compelling evidence of heterologous protection and open a promising route to the improvement of the elusive broadly protective PRRS vaccine.

60 Leng, C., Zhang, W., Zhang, H., Kan, Y., Yao, L., Zhai, H., ... & Peng, J. (2017). ORF1a of highly pathogenic PRRS attenuated vaccine virus plays a key role in neutralizing antibody induction in piglets and virus neutralization in vitro. *Virology Journal*, 14(1), 159.

61 Vu, H. L., Pattnaik, A. K., & Osorio, F. A. (2017). Strategies to broaden the cross-protective efficacy of vaccines against porcine reproductive and respiratory syndrome virus. *Veterinary Microbiology*, 206, 29-34.

Improved vaccine against PRRSV: current progress and future perspective (Nan et al., 2017[62])

Current strategies for controlling PRRS virus (PRRSV) are inadequate, also due to the constant emergence of new PRRSV variants. In addition, numerous safety and efficacy concerns for currently licensed vaccines (e.g. shedding of modified live virus, MLV; reversion to virulence; recombination between field strains and MLV; failure to elicit protective immunity against heterogeneous virus) have been reported, highlighting the need for effective PRRS vaccines. This article, published on Frontiers in microbiology, reviews recent advances in PRRSV vaccine development. The paper presents detailed discussions on antigenic variations resulting from PRRSV evolution, identification of neutralising epitopes for heterogeneous isolates, broad neutralising antibodies against PRRSV, chimeric virus generated by reverse genetics, and novel PRRSV strains with interferon-inducing phenotype. Moreover, the authors focus on techniques that could potentially transform current MLV vaccines into a superior vaccine and on new insights for future PRRSV vaccine development. In the view of the authors, improved PRRSV vaccines may overcome the disadvantages of current vaccines and minimise the PRRS impact to the swine industry.

Exosomes mediate intercellular transmission of porcine reproductive and respiratory syndrome virus (Wang et al., 2017[63])

This article investigates the role of exosomes in PRRS virus (PRRSV) infection, to clarify if they mediate intercellular transmission of PRRSV. The authors found that purified exosomes isolated from PRRSV-infected cells contained viral genomic RNA and partial viral proteins. Furthermore, exosomes from PRRSV-infected cells established productive infection in both PRRSV-susceptible and -nonsusceptible cells. More importantly, exosome-mediated infection was not completely blocked by PRRSV-specific neutralising antibodies. The authors concluded that, based on these findings, exosomes can mediate PRRSV transmission and are even resistant to antibody neutralisation, identifying a potential immune evasion mechanism used by PRRSV.

Identification of resilient sows in Porcine Reproductive and Respiratory Syndrome virus infected farms (Abella et al., 2019[64])

In this paper, published in the Journal of Animal Science, Abella and colleagues aimed to develop a phenotyping criterion to discriminate susceptible from resilient sows in farms infected with porcine reproductive and respiratory syndrome virus (PRRSV). A total of 517 Landrace x Large White gilts were classified as resilient (R) or susceptible (S) to PRRSV virus, following vaccination with MLV-PRRSV at 6-7 wk of age, in a PRRSV negative multiplication farm. Female piglets were phenotyped as R if their serum was negative to PRRSV at 7 and 21 d post-vaccination (DPV) or as S if their serum was positive at 7 and/or 21 DPV. Amongst them, 382 gilts were transferred to a PRRSV-positive production farm, where the number of piglets born alive (NBA), stillborn (NSB), mummified (NMU), lost (NLP=NSB+NMU) and total born (NTB = NBA+NLP) were recorded for almost three years. Data were collected during two periods according to the PRRSV farm health status, which were confirmed as either PRRSV-positive stable (endemic) or instable (epidemic). The heritability of the resilience criterion was 0.46 (SD 0.06). The probability of a piglet being lost was greater (≥0.97) in S than in R litters, regardless of whether the delivery occurred during a PRRSV outbreak (20.5% vs 17.0%) or not (15.8% vs 13.7%). The lower piglet mortality rate in R sows was due to NSB, in the endemic phase (13.0% vs 15.0% of NTB, with a posterior probability of 98% of S sows showing higher NSB than R sows), and to NMU, in the epidemic phase (4.0% vs 8.4% of NTB, with a posterior probability of >99% of S sows showing higher NMU than R

62 Nan, Y., Wu, C., Gu, G., Sun, W., Zhang, Y. J., & Zhou, E. M. (2017). Improved vaccine against PRRSV: current progress and future perspective. *Frontiers in Microbiology*, *8*, 1635.

63 Wang, T., Fang, L., Zhao, F., Wang, D., & Xiao, S. (2017). Exosomes mediate intercellular transmission of porcine reproductive and respiratory syndrome virus (PRRSV). *Journal of Virology*, JVI-01734.

64 Abella, G., Novell, E., Tarancon, V., Varona, L., Pena, R. N., Estany, J., & Fraile, L. (2019). Identification of resilient sows in Porcine Reproductive and Respiratory Syndrome virus infected farms. *Journal of animal science*, 97(8), 3228–3236.

sows). During a PRRSV outbreak, the S sows were twice as likely to give birth to a mummified piglet as compared to R sows. These findings provided evidence that the described phenotyping scheme has a potential use as a PRRSV resilience criterion.

Establishment of systems to enable isolation of porcine monoclonal antibodies broadly neutralizing the porcine reproductive and respiratory syndrome virus (Goldeck et al., 2019[65])

In this article, published in Frontiers in Immunology, the authors sought to establish systems to enable the isolation of porcine reproductive and respiratory syndrome viruses (PRRSV) neutralising porcine monoclonal antibodies (mAbs), to be used for the discovery of new targets for next-generation vaccines conferring heterologous protection. A cohort of immune pigs was produced by sequential challenge infection with four heterologous PRRSV strains spanning PRRSV-1 subtypes and PRRSV species. Whilst priming with PRRSV-1 subtype 1 did not confer full protection against a subsequent infection with a PRRSV-1 subtype 3 strain, animals were protected against a subsequent PRRSV-2 infection. The infection protocol resulted in high serum neutralising antibody titres against PRRSV-1 Olot/91 and significant neutralisation of heterologous PRRSV-1/-2 strains. Enriched memory B cells isolated at the termination of the study were genetically programmed by transduction with a retroviral vector expressing the Bcl-6 transcription factor and the anti-apoptotic Bcl-xL protein, a technology that appeared to efficiently convert porcine memory B cells into proliferating antibody-secreting cells. Pools of transduced memory B cells were cultured and supernatants containing PRRSV-specific antibodies identified by flow cytometric staining of infected MARC-145 cells and in vitro neutralisation of PRRSV-1. The authors concluded that these data suggest that this experimental system may be further exploited to produce a panel of PRRSV-specific mAbs, which will contribute both to our understanding of the antibody response to PRRSV and allow epitopes to be resolved that may ultimately guide the design of immunogens to induce cross-protective immunity.

Key gaps in the knowledge of the porcine respiratory reproductive syndrome virus (PRRSV) (Montaner-Tarbes et al., 2019[66])

A review, published in Frontiers in Veterinary Science, outlines key gaps in the knowledge of the porcine respiratory reproductive syndrome virus (PRRSV). PRRSV displays complex interactions with the immune system and a high mutation rate, making the development, and implementation of control strategies a major challenge. The review addresses the biology of the virus, focussing on newly discovered functions of non-structural proteins and novel dissemination mechanisms. Secondly, it reviews the role of different cell types and viral proteins in natural and vaccine-induced immune responses together with the role of different immune evasion mechanisms focusing on those gaps of knowledge that are critical to generate more efficacious vaccines. Finally, it discusses novel strategies for antigen discovery and vaccine development, in particular the use of exosomes (extracellular vesicles of endocytic origin).

7. Coronaviruses

Global network

The coronaviral diseases of interest for the STAR-IDAZ IRC are infectious bronchitis, severe acute

65 Goldeck, D., Perry, D. M., Hayes, J. W., Johnson, L. P., Young, J. E., Roychoudhury, P., ... & Frossard, J. P. (2019). Establishment of systems to enable isolation of porcine monoclonal antibodies broadly neutralizing the porcine reproductive and respiratory syndrome virus. *Frontiers in Immunology*, 10.

66 Montaner-Tarbes, S., del Portillo, H. A., Montoya, M., & Fraile, L. (2019). Key gaps in the knowledge of the Porcine Respiratory Reproductive Syndrome Virus (PRRSV). *Frontiers in veterinary science*, 6.

respiratory syndrome (SARS), MERS and swine enteric coronaviruses, including porcine epidemic diarrhoea (PED), transmissible gastroenteritis (TGE), and a new bat-HKU2-like porcine coronavirus.

No global network on coronaviruses has been formed yet. Due to the width of the topic, that involves several diseases affecting different animal species, as well as humans (e.g. MERS). SIRCAH is currently working to better define the scope of a possible STAR-IDAZ IRC Working Group (WG) on the topic, following the procedures indicated in the Terms of Reference for establishing WGs. In preparation for the drafting of roadmaps for coronaviruses, the current state of the art for vaccines and diagnostics was mapped by a member of the STAR-IDAZ IRC Scientific Committee. SIRCAH will ask the support of experts for revising the document.

DISCONTOOLS

The database does not contain information on corona viral diseases.

Recent developments

Synthetic virus-like particles prepared via protein corona formation enable effective vaccination in an avian model of coronavirus infection (Chen et al., 2016[67])

An article published in Biomaterials describes the development of new vaccine formulations with virus-like features, which enhance antigen presentation and immune processing, as to improve the control of coronavirus infection. Using an avian coronavirus spike protein as a model antigen, synthetic virus-like particles (sVLPs) were prepared by incubating 100 nm gold nanoparticles in a solution containing an optimised concentration of viral proteins. Following removal of free proteins, antigen-laden particles were recovered and showed morphological semblance to natural viral particles under nanoparticle tracking analysis and transmission electron microscopy. As compared to inoculation with free proteins, vaccination with the sVLPs showed enhanced lymphatic antigen delivery, stronger antibody titres, increased splenic T-cell response, and reduced infection-associated symptoms in an avian model of coronavirus infection. The authors compared the new vaccine to a commercial whole inactivated virus vaccine, showing evidence of superior antiviral protection by the sVLPs. The study demonstrates a simple yet robust method in bridging viral antigens with synthetic nanoparticles for improved vaccine application. The authors concluded that these results would have practical implications in the management of human as well as animal viral infections.

Protection against infectious bronchitis virus by spike ectodomain subunit vaccine (Eldemery et al., 2017[68])

This article, published in Vaccine, investigated the use of novel antigens for producing more effective vaccines against infectious bronchitis virus (IBV) in chicken. The (IBV) S1 subunit of the spike (S) glycoprotein mediates viral attachment to host cells and the S2 subunit is responsible for membrane fusion. Although the S1 subunit is the major inducer of neutralising antibodies, vaccination with S1 protein has been shown to confer inadequate protection against challenge. Using IBV Arkansas-type (Ark) S protein histochemistry, the authors showed that extension of S1 with the S2 ectodomain improves binding to chicken tissues. The demonstrated contribution of S2 ectodomain to binding to chicken tissues suggests that vaccination with the ectodomain might improve protection compared to vaccination with S1 alone. In this study, after immunisation with recombinant trimeric soluble

67 Chen, H. W., Huang, C. Y., Lin, S. Y., Fang, Z. S., Hsu, C. H., Lin, J. C., ... & Hu, C. M. J. (2016). Synthetic virus-like particles prepared via protein corona formation enable effective vaccination in an avian model of coronavirus infection. *Biomaterials*, *106*, 111-118.

68 Eldemery, F., Joiner, K. S., Toro, H., & van Santen, V. L. (2017). Protection against infectious bronchitis virus by spike ectodomain subunit vaccine. *Vaccine*, 35(43), 5864-5871.

IBV Ark-type S1 or S-ectodomain protein, followed by challenge with virulent Ark IBV 21 days after boost, chickens immunised with recombinant S-ectodomain protein showed statistically significantly reduced viral loads compared to chickens immunised with recombinant S1 protein. Consistent with viral loads, significantly reduced tracheal mucosal thickness and tracheal lesion scores were observed in recombinant S-ectodomain vaccinated animals. These results indicate that the S2 domain has an important role in inducing protective immunity. The authors suggested that including the S2 domain with S1 might be promising for better viral vectored and/or subunit vaccine strategies.

Vaccination against infectious bronchitis virus: A continuous challenge (Jordan, 2017[69])

This review, published in Veterinary Microbiology, outlines the current situation as it relates to control of Infectious bronchitis virus (IBV). While either live-attenuated or killed vaccines exists and are used extensively, novel vaccines are needed to combat emerging and variant IBV serotypes. The article describes current advances toward the development of subunit, peptide, DNA, and recombinant vaccines (including also recombinant IBV generated by reverse genetics), that may provide better protection in the future. The authors also discuss other factors that can influence the outcome of vaccination (e.g. application method, vaccine combinations, chicken genetics, and immune responses), that need to be taken into account when developing vaccines.

Porcine deltacoronavirus infection: Etiology, cell culture for virus isolation and propagation, molecular epidemiology and pathogenesis (Jung et al., 2016[70])

Porcine deltacoronavirus (PDCoV) is a novel swine enteropathogenic coronavirus causing acute diarrhoea/vomiting, dehydration and mortality in seronegative neonatal piglets, that is causing high mortality and economic losses in the United States. This review, published in Virus research, focuses on the current knowledge on aetiology, cell culture isolation and propagation, molecular epidemiology, disease mechanisms and pathogenesis of PDCoV infection.

S1 domain of the porcine epidemic diarrhea virus spike protein as a vaccine antigen (Makadyia et al., 2016[71])

An article published in the Virology journal investigates the use of the N-terminal subunit of spike protein (S1), which is responsible for PED virus (PEDV) binding to the cellular receptor and contains several neutralising antibody epitopes, to protect new-born piglets by immunisation of sows. The authors expressed and produced recombinant PEDV S1 protein and immunised a pregnant sow intramuscularly three times with adjuvanted recombinant protein prior to farrowing. PEDV-specific immune responses in sera and colostrum of the sow and piglets were assayed by ELISA and virus neutralisation assays. Piglets were challenged orally with PEDV, and clinical parameters were monitored for 6 days post-challenge. The administration of the subunit vaccine in the sow resulted in induction of S1-specific IgG and IgA that were passively transferred to the suckling piglets. Also, high virus neutralisation titres were observed in the serum of the vaccinated sow and its piglets. After PEDV challenge, piglets born to the vaccinated sow exhibited less severe signs of disease and significantly lower mortality compared to the piglets of a control sow. However, there were no significant differences in diarrhoea, body weight and virus shedding. Thus, vaccination with S1 subunit vaccine failed to provide complete protection to suckling piglets after challenge exposure, and further improvements are needed for the development of a subunit vaccine that fully protects against PEDV infection.

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69 Jordan, B. (2017). Vaccination against infectious bronchitis virus: A continuous challenge. *Veterinary Microbiology*, 206, 137-143.

70 Jung, K., Hu, H., & Saif, L. J. (2016). Porcine deltacoronavirus infection: etiology, cell culture for virus isolation and propagation, molecular epidemiology and pathogenesis. *Virus Research*, *226*, 50-59.

71 Makadiya, N., Brownlie, R., van den Hurk, J., Berube, N., Allan, B., Gerdts, V., & Zakhartchouk, A. (2016). S1 domain of the porcine epidemic diarrhea virus spike protein as a vaccine antigen. *Virology Journal*, *13*(1), 57.

Vaccines for porcine epidemic diarrhea virus and other swine coronaviruses (Gerdts & Zakhartchouk, 2017[72])

An article published in Viruses reviews existing vaccine technologies for swine coronaviruses and highlights promising technologies which may help to control these important viruses in the future. The review mostly focuses on PED (in Asia and North America) but also covers transmissible gastroenteritis virus (TGEV) and other coronaviruses (e.g. deltacoronaviruses), also highlighting future perspectives for pig coronavirus vaccines.

Immunogenicity of eGFP-Marked Recombinant Lactobacillus casei against Transmissible Gastroenteritis Virus and Porcine Epidemic Diarrhea Virus (Yu et al., 2017[73])

This article describes a study aiming to develop an effective oral bivalent vaccine for porcine transmissible gastroenteritis virus (TGEV) and porcine epidemic diarrhoea virus (PEDV). The D antigenic site of the TGEV spike (S) protein and the major antigen site (core neutralising epitope—COE) of the PEDV S protein were used as immunogens, and the enhanced green fluorescent protein (eGFP) gene was used as a reporter to construct genetically engineered Lactobacillus casei rLpPGF-T7g10-eGFP-6D-COE. The expression of proteins of interest by the recombinant L. casei was confirmed by confocal laser scanning microscopy and a Western blot assay, and the immunogenicity of rLpPGF-T7g10-eGFP-6D-COE was evaluated in orally immunised mice. The levels of anti-PEDV and anti-TGEV serum immunoglobulin G (IgG) and mucosal secreted immunoglobulin A antibodies obtained from the mice immunised with rLpPGF-T7g10-eGFP-6D-COE, as well as the proliferation levels of lymphocytes, were significantly higher than those in mice orally administered phosphate-buffered saline (PBS) or rLpPG-T7g10. Moreover, the serum IgG showed neutralising effects against PEDV and TGEV. The authors concluded that the antibiotic resistance-free genetically engineered L. casei bivalent oral vaccine provides a safe and promising strategy for vaccine development against PEDV and TGEV.

Fatal swine acute diarrhoea syndrome caused by an HKU2-related coronavirus of bat origin (Zhou et al., 2018[74])

An article published in Nature provides evidence that a novel HKU2-related bat coronavirus, swine acute diarrhoea syndrome coronavirus (SADS-CoV), has been the aetiological agent of a large-scale outbreak of fatal disease in pigs in China that has caused the death of 24,693 piglets across four farms. SADS-related CoVs with 96–98% sequence identity were found in 9.8% (58 out of 591) of anal swabs collected from bats in Guangdong province during 2013–2016, predominantly in horseshoe bats (Rhinolophus spp.), that are known reservoirs of severe acute respiratory syndrome (SARS) related CoVs. Bats have been recognised as one of the most important reservoirs for emerging viruses and the transmission of a coronavirus that originated in bats to humans via intermediate hosts was responsible for the SARS epidemic. The researchers found striking similarities between the SADS and SARS outbreaks in geographical, temporal, ecological and aetiological settings. This study highlights the importance of identifying coronavirus diversity and distribution in bats to mitigate future outbreaks that could threaten livestock, public health and economic growth.

72 Gerdts, V., & Zakhartchouk, A. (2017). Vaccines for porcine epidemic diarrhea virus and other swine coronaviruses. *Veterinary Microbiology*, 206, 45-51.

73 Yu, M., Wang, L., Ma, S., Wang, X., Wang, Y., Xiao, Y., ... & Li, Y. (2017). Immunogenicity of eGFP-marked recombinant Lactobacillus casei against transmissible gastroenteritis virus and porcine epidemic diarrhea virus. *Viruses*, *9*(10), 274. Yu, M., Wang, L., Ma, S., Wang, X., Wang, Y., Xiao, Y., ... & Li, Y. (2017). Immunogenicity of eGFP-marked recombinant Lactobacillus casei against transmissible gastroenteritis virus and porcine epidemic diarrhea virus. *Viruses*, *9*(10), 274.

74 Zhou, P., Fan, H., Lan, T., Yang, X. L., Shi, W. F., Zhang, W., ... & Zheng, X. S. (2018). Fatal swine acute diarrhoea syndrome caused by an HKU2-related coronavirus of bat origin. *Nature*, 556(7700), 255.

Livestock susceptibility to infection with Middle East respiratory syndrome coronavirus (Vergara-Alert et al., 2017[75])

In this article, published in Emerging Infectious Diseases, the author investigated whether other animals than dromedaries are potential reservoirs for Middle East respiratory syndrome (MERS). MERS coronavirus (MERS-CoV) was inoculated into llamas, pigs, sheep, and horses and nasal and rectal swab samples were collected at various times. The presence of MERS-CoV in the nose of pigs and llamas was confirmed by PCR, titration of infectious virus, immunohistochemistry, and in situ hybridisation; seroconversion was detected in animals of both species. Conversely, in sheep and horses, no virus-specific antibodies were detected and no evidence of viral replication in the upper respiratory tract was found. These results prove the susceptibility of llamas and pigs to MERS-CoV infection. The authors concluded that the possibility of MERS-CoV circulation in animals other than dromedaries, such as llamas and pigs, is not negligible.

75 Vergara-Alert, J., van den Brand, J. M., Widagdo, W., & Muñoz, M. (2017). Livestock susceptibility to infection with Middle East respiratory syndrome coronavirus. *Emerging Infectious Diseases*, 23(2), 232.

A Recombinant Newcastle Disease Virus (NDV) Expressing S Protein of Infectious Bronchitis Virus (IBV) Protects Chickens against IBV and NDV (Shirvani et al., 2018[76])

This study published in Scientific Reports, describes the development of a recombinant Newcastle disease virus (NDV) expressing S1, S2 and S proteins of infectious bronchitis virus (IBV). Results showed that the rNDV expressing the S protein of IBV provided better protection than the rNDV expressing S1 or S2 protein of IBV, indicating that the S protein is the best protective antigen of IBV. Immunisation of 4-week-old SPF chickens with the rNDV expressing S protein elicited IBV-specific neutralising antibodies and provided complete protection against virulent IBV and virulent NDV challenges. These results suggest that the rNDV expressing the S protein of IBV is a safe and effective bivalent vaccine candidate for both IBV and NDV.

Recombinant Chimeric Transmissible Gastroenteritis Virus (TGEV)—Porcine Epidemic Diarrhea Virus (PEDV) Virus Provides Protection against Virulent PEDV (Pascual-Iglesias et al., 2019[77])

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.

Adaptive Evolution of MERS-CoV to Species Variation in DPP4 (Letko et al., 2018[78])

An article, appeared in Cell Reports, investigated the genetic mechanisms underlying cross-species adaptation of Middle East Respiratory Syndrome Coronavirus (MERS-CoV). Variation in the host receptor, dipeptidyl peptidase 4 (DPP4), can block the interaction with the MERS-CoV spike protein and form a species barrier to infection. To better understand the species adaptability of MERS-CoV, the authors identified a suboptimal species-derived variant of DPP4 to study viral adaption. Passaging virus on cells expressing this DPP4 variant led to accumulation of mutations in the viral spike which increased replication. Parallel passages revealed distinct paths of viral adaptation to the same DPP4 variant. Structural analysis and functional assays showed that these mutations enhanced viral entry with suboptimal DPP4 by altering the surface charge of spike. These findings demonstrated that MERS-CoV spike can utilise multiple paths to rapidly adapt to novel species variation in DPP4.

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76 Shirvani, E., Paldurai, A., Manoharan, V. K., Varghese, B. P., & Samal, S. K. (2018). A recombinant Newcastle disease virus (NDV) Expressing S protein of infectious bronchitis virus (IBV) protects chickens against IBV and NDV. *Scientific reports*, 8(1), 11951.

77 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.

78 Letko, M., Miazgowicz, K., McMinn, R., Seifert, S. N., Sola, I., Enjuanes, L., ... & Munster, V. (2018). Adaptive evolution of MERS-CoV to species variation in DPP4. *Cell reports*, 24(7), 1730-1737.

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, that 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. A list of possible members to form the WG is under consideration.

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.

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.