STAR-IDAZ
International Research
Consortium on Animal Health

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

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Index

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

Disclaimer:

The report is a presentation of the current initiatives and recent scientific literature, organised to identify and highlight trends and advances in research on animal health, with a focus on priority animal diseases at a global level. The report does not target initiatives aimed at implementing animal disease control strategies (e.g. roadmaps for the control or eradication of infectious diseases) nor 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 at the international level research activities in an effort 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/strategies, including candidate vaccines, diagnostics, therapeutics and other animal health products and procedures and/or key scientific information/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 should support the decisions of policy makers and research funders, so as 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 already available knowledge, and for modelling disease impact. Several initiatives have been started, at regional or at 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 fast track the 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 an initial investment of US $540 million from the governments of Germany, Japan and Norway, plus the Bill & Melinda Gates Foundation and Wellcome. The European Commission and India also plan to invest additional funding in the initiative. CEPI has also a secretariat, supported by the governments of Norway and India, Wellcome, the Bill & Melinda Gates Foundation, and the World Economic Forum.

CEPI activities will 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 yearly updated.

Although CEPI focus is on human diseases, most of the diseases in the WHO R&D Blueprint are zoonoses, and, in some specific cases, CEPI might consider the development of animal vaccines, as this would represent an added value for the control of the disease, preventing the development of human cases.

The first diseases that will be targeted by CEPI are those caused by Middle East Respiratory Syndrome coronavirus (MERS-CoV), Lassa virus and Nipah virus.

Global Challenges Research Fund (GCRF)

http://www.rcuk.ac.uk/funding/gcrf/

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.

The first calls closed in 2016 and already targeted several issues of interest to the STAR-IDAZ IRC priorities. As of 2017, several calls are open and will be closed by autumn; the selected project will most likely start by 2018.

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. Its focus is on livestock diseases of major economic importance to small-scale livestock producers, including such diseases as East Coast Fever, Newcastle Disease, Contagious Bovine Pleuropneumonia and Peste des Petits Ruminants.

GALVmed was formally established in 2005, with initial funding from the UK Government. 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.

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.

Dedicated programmes have targeted African trypanosomosis, Contagious Bovine Pleuropneumonia (CBPP), brucellosis, East Coast Fever (ECF), while other programmes in general target neglected animal diseases in Africa.

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.

Pull mechanisms such as this prize, rewarding research output rather than research input, represents a good way forward 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 focus 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 might indicate 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, within a few months after the occurrence of first cases, to major new infectious disease threats at the European and global levels by designing new manufacturing processes (up to large scale) for delivering effective control tools (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 neutralizing antibodies against potential new zoonotic diseases or strains, 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.

The project will mainly focus on three key viruses: Rift Valley fever virus (RVF), Schmallenberg virus and Middle East respiratory syndrome coronavirus (MERS-CoV).

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, commercialize, and deploy livestock vaccines.

II. INTERNATIONAL INITIATIVES TO FACILITATE TRANSNATIONAL COLLABORATIONS

The progressive reduction of public funding, as well as the increasing need of preparedness for emerging diseases, creates the pressing need to prioritise research needs and to prevent unnecessary duplication. 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 to improve 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 three 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), and the ANIHWA ERA Net (European Research Area Network on Animal Health and Welfare).

The CWG is still running, and has been holding 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)

(website not yet available)

The European Joint Programme (EJP) Co-fund on One Health (zoonoses – emerging threats) has been selected for funding under the EU H2020 Programme in 2017. It is expected that the grant agreement will be signed by the end of 2017 and the project should start at the beginning of 2018.

This 5 year (2018-2023), 90 million euros, initiative will aim to create a European joint programme to deal with "one health", primarily targeting food-borne zoonoses and antimicrobial resistance, and to a lesser extent emerging zoonotic threats. The project Consortium includes 43 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 will aim 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 list of research topics was selected by the Consortium before the submission of the proposal. In addition to traditional research projects, the EJP will fund 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

(website for the specific networks 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 Foot and Mouth Disease, African Swine Fever, animal influenza, helminths, and bovine tuberculosis. 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 will facilitate 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 around 20 industrial and academic partners, and is looking to enlarge its membership.

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 AfVANET plans to organise a first meeting in Africa in 2017, with support from international donors and intergovernmental organisations (including the OIE, the FAO and the WHO).

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.

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/

The DISCONTOOLS (DISease CONtrol TOOLS) project was funded under the European Commission FP7 (2008-2013) with the objective to evaluate global animal health research priorities, assess the risk they pose to the EU, and guide the allocation of research funds. The project led to the development of a disease database that includes the results of gap analyses and a prioritisation model for 52 infectious animal diseases. A dedicated expert group was convened for each disease.

Since the end of the project funding under FP7, DISCONTOOLS is funded by a consortium of EU Member States, with industry providing secretariat support, with the aim to keep the database updated. As such, the database has become an important resource for funders of animal health research when developing their research agendas, specific calls for research and for the evaluation of submitted research proposals. The research gap analyses delivered by DISCONTOOLS were used as the starting points for the STAR-IDAZ gap analyses and will continue to be used in this way for the STAR-IDAZ IRC.

For each disease, the expert groups delivered several documents, which include the description of the disease and the related research gaps ("Disease details"), the list of related control tools ("Control tools") and two scoring models ("Gap analysis" and "Disease prioritisation model"). The website is currently being re-developed to improve the user interface and to facilitate consultation in research gap analyses.

Veterinary Biocontained research facility Network (VetBioNet)

http://www.vetbionet.eu/

VetBioNet (Veterinary Biocontained Research Facility Network) is a project funded under the European Commission Horizon 2020 Research Framework for large research infrastructures (2017-2021). The project consortium includes 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 among the 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 25 laboratories, 16 belonging to EU member state institutions and 9 to non-EU institutions, and a number of Associated Partners (to date, 14 institutions from 11 non-EU member states and 3 EU member states), all sharing the common interest of creating an international virus collection.

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.

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
- Innovative anti-infective approaches, including alternatives to anti-microbial agents
- 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 topics to be addressed were selected. These are: ASF, bTB, brucellosis, FMD, helminths, and PRRS.

This section of the report will focus on the topics selected to be addressed first. In the yearly update of the report, information on the diseases, that are already present, will be updated and sections on the newly selected diseases will be developed.

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

1. Existing or planned global networks that aim at guiding future research on the topic, and that will act 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 will be 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 will be 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 will form SIRCAH 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 and have selected key articles reviewing the current state of knowledge, or providing significant advances in the science. For most of the priority diseases, it was not feasible to include a comprehensive list of recent publications in this report due to the large volume of literature published on them.

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 this first 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, 34 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 anopportunity for researchers to network and exchange new knowledge about the disease and the development of control tools.

The next meeting will be held in Cagliari (Italy) in April 2018. The meeting will be organised in collaboration with the GARA, STAR-IDAZ IRC and the local hosting institution (Istituto Zooprofilattico Sperimentale della Sardegna).

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. There is little commercial interest in licensing of new vaccines due to potential demand. 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.

Recent developments

ASF vaccine development: new target identified for gene deletion (O'donnell et al., 2015[1])

An article published in the Journal of Virology describes the construction, and test as a candidate vaccine, of a recombinant virus (ASFV-G-ΔMGF) derived from the highly virulent ASFV Georgia 2007 isolate (ASFV-G) by specifically deleting six genes belonging to Multigene family 360 (MGF360) or and 505 (MGF505). These MGFs are a group of genes sharing partial sequence and structural identities that have been connected with ASFV host range specificity, blocking of the host innate response, and virus virulence. The new virus gave good results in vitro, replicating as efficiently in primary swine macrophage cell cultures as the parental virus. In vivo, it was completely attenuated, with inoculated pigs remaining healthy, and it was effective in preventing sign of disease on pigs after challenge with highly virulent parental ASFV-G, although a proportion of these animals harboured the challenge virus. The authors declare this to be the first experimental vaccine reported to induce protection in pigs challenged with highly virulent and epidemiologically relevant ASFV-G.

[1] O'donnell, V., Holinka, L. G., Gladue, D. P., Sanford, B., Krug, P. W., Lu, X., Arzt, J., Reese, B., Carrillo, C., Risatti, G. R., & Borca, M. V. (2015). African swine fever virus Georgia isolate harboring deletions of MGF360 and MGF505 genes is attenuated in swine and confers protection against challenge with virulent parental virus. Journal of virology, 89(11), 6048-6056.

Some serotype-specific proteins are significant ASF protective antigens (Burmakina et al., 2016[2])

An article published in the Journal of General Virology investigated if some serotype-specific proteins are significant protective antigens for ASF. Based on the fact that recent data showed that ASF protective immunity may be haemadsorption inhibition (HAI) serotype-specific, the authors produced ASFV inter-serotypic chimeric viruses using two proteins which are necessary and sufficient for mediating HAI serological specificity (CD2v and C-type lectin). The chimeric viruses were successfully used for vaccination/challenge trials in pigs, demonstrating that serotype-specific proteins are important for protection against homologous ASFV infection. The authors conclude that these viral proteins represent significant protective antigens for ASFV, and suggest that these should be targeted in future vaccine design and development.

Use of cross-priming amplification method for ASF diagnosis (Frączyk et al., 2016[3])

An article published in Letters in Applied Microbiology describes the development of cross-priming amplification (CPA) for direct detection of the ASF virus in pig and wild boar blood and sera samples. The CPA specifically targets ASF virus DNA only. Study results showed that the CPA had equal sensitivity in comparison to the official real-time PCR. The developed method was able to detect ASF virus in all examined blood samples, both from pigs and wild boar. According to the authors, the developed methodology might be further used by local and county veterinary officers, hunters or pig farmers, for preliminary ASF diagnosis.

Is deletion of interferon inhibitors a possible route for producing ASF attenuated vaccines? (Reis et al., 2016[4])

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

[2] Burmakina, G., Malogolovkin, A., Tulman, E. R., Zsak, L., Delhon, G., Diel, D. G., Shobogorov, N. M., Morgunov, Yu. P., Morgunov, S. Yu., Kutish, G. F., Kolbasov, D., & Rock D. L. (2016). African swine fever virus serotype-specific proteins are significant protective antigens for African swine fever. Journal of General Virology, 97(7), 1670-1675.

[3] Frączyk, M., Woźniakowski, G., Kowalczyk, A., Niemczuk, K., & Pejsak, Z. (2016). Development of cross-priming amplification for direct detection of the African Swine Fever Virus, in pig and wild boar blood and sera samples. Letters in applied microbiology, 62(5), 386-391

4 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

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 concluded that the deletion of IFN modulators would be a promising route for the construction of rationally attenuated ASFV candidate vaccine strains.

Novel ASF detection method in blood (Sastre et al., 2016[5])

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[6])

An article published in the Archives of virology describes the development of a novel PCR assay for detection of African Swine Fever 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.

[5] 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.

[6] 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[7])

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 African Swine Fever 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.

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 will be evaluated and validated by bTB experts at a workshop in the United Kingdom in December and submitted to GRAbTB for approval. The roadmaps will then be presented

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

by SIRCAH for endorsement by the STAR-IDAZ IRC members and consideration as to how they might be taken forward.

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

Identification and evaluation of new *Mycobacterium bovis* antigens in the in vitro interferon gamma release assay for bovine tuberculosis diagnosis (Eirin et al., 2015[8])

In their article published in Tuberculosis, Eirin and colleagues described the identification and evaluation of new *M. bovis* antigens, to be used as alternatives to the protein purified derivative tuberculin (PPD), which is considered a complex and poorly characterised reagent. Six *M. bovis* Open Reading Frames (Mb1992, Mb2031c, Mb2319, Mb2843c, Mb2845c and Mb3212c) were selected and evaluated in experimental and natural infection. The reactivity performance was tested in animals with both positive and negative Tuberculin Skin Test (TST) results, as well as in cattle infected with *Mycobacterium avium subsp. paratuberculosis* (MAP). The six recombinant antigens individually induced an IFN-γ response. Mb2845c was the most valuable antigen with the potential to discriminate TST-positive cattle from either TST-negative or MAP infected animals. The presented data confirmed the feasibility to implement bioinformatic screening tools and suggested Mb2845c as a potential diagnostic antigen, to be tested in protein cocktails to evaluate their contribution to bTB diagnosis.

Use of bacterial whole-genome sequencing to investigate local persistence and spread in bovine tuberculosis (Trewby et al., 2016[9])

In this article, published in Epidemics, Trewby and collaborators described the application of bacterial whole-genome sequencing (WGS) to investigate local persistence and spread in bovine tuberculosis. The

[8] Eirin, M. E., Macias, A., Magnano, G., Morsella, C., Mendez, L., Blanco, F. C., ... & Singh, M. (2015). Identification and evaluation of new Mycobacterium bovis antigens in the in vitro interferon gamma release assay for bovine tuberculosis diagnosis. Tuberculosis, 95(6), 795-801.

[9] Trewby, H., Wright, D., Breadon, E. L., Lycett, S. J., Mallon, T. R., McCormick, C., ... & Herzyk, P. (2016). Use of bacterial whole-genome sequencing to investigate local persistence and spread in bovine tuberculosis. Epidemics, 14, 26-35.

study was conducted on a sub-population of *M. bovis* in 145 cattle across 66 herd breakdowns. Despite the low divergence among isolates, WGS seemed to allow exposing contributions of under-sampled host populations to *M. bovis* transmission. The authors found that isolates from farms with a known history of direct cattle movement between them showed a statistical signal of higher genetic similarity and that genetic distances showed no apparent relationship with spatial distance among affected farms over distances <5 kilometres. Using simulations, the authors found that Bayesian phylogeographic approaches were feasible, and applied them, showing that *M. bovis* dispersal in this system was heterogeneous but slow overall, averaging 2 km/year. Trewby and colleagues concluded that that widespread application of WGS to *M. bovis* would bring novel and important insights into the dynamics of *M. bovis* spread and persistence, but that the current questions most pertinent to control would be best addressed using approaches that more directly integrate WGS with additional epidemiological data.

Bovine tuberculosis in cattle: vaccines, DIVA tests, and host biomarker discovery (Vordermeier et al., 2016[10])

In this review, published in Annual review of animal biosciences, the authors described recent advances on bTB vaccination. The paper covered three main areas: (i) progress made on optimising the only potentially available vaccine, bacille Calmette Guérin (BCG), and on strategies to improve BCG efficacy; (ii) recent advances in DIVA development based on the detection of host cellular immune responses by blood-testing or skin-testing approaches; and (iii) the definition of host biomarkers that provide meaningful stage-gating criteria to select vaccine candidates for further testing.

Interleukin-17A as a biomarker for bovine tuberculosis (Waters et al., 2016[11])

In this paper, Waters and colleagues evaluated interleukin-17 (IL-17) biology in the context of *M. bovis* infection of cattle. T-helper 17 (Th17)-associated cytokines are integral to the immune responses to tuberculosis. Using transcriptome sequencing (RNA-Seq), numerous Th17-associated cytokine genes (including IL-17A, IL-17F, IL-22, IL-19, and IL-27) were upregulated in response to purified protein derivative stimulation of peripheral blood mononuclear cells from experimentally *M. bovis*-infected cattle. Protective vaccines elicited IL-17A, IL-17F, IL-22, and IL-27 responses. Reduced IL-17A responses by vaccine recipients, compared to non-vaccinated animals, at 2.5 weeks after *M. bovis* challenge correlated with reduced disease burdens. Additionally, IL-17A and interferon gamma (IFN-γ) responses were highly correlated and exhibited similar diagnostic capacities. The authors concluded that these results supported the use of Th17-associated cytokines as biomarkers of infection and protection in the immune responses to bTB.

[10] Vordermeier, H. M., Jones, G. J., Buddle, B. M., Hewinson, R. G., & Villarreal-Ramos, B. (2016). Bovine tuberculosis in cattle: vaccines, DIVA tests, and host biomarker discovery. Annual review of animal biosciences, 4, 87-109.
[11] Waters, W. R., Maggioli, M. F., Palmer, M. V., Thacker, T. C., McGill, J. L., Vordermeier, H. M., ... & Larsen, M. H. (2016). Interleukin-17A as a biomarker for bovine tuberculosis. Clinical and Vaccine Immunology, 23(2), 168-180.

Genetic evaluation for bovine tuberculosis resistance in dairy cattle (Banos et al., 2017[12])

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

3. Brucellosis

Global network

Under the STAR-IDAZ project, an expert group on brucellosis had been formed and, in 2014, conducted, a first research gap analysis. No formal group has since been established, but the IRC Secretariat is currently working to expand the initial list of experts as to establish a STAR-IDAZ IRC Working Group (WG), following the procedures indicated in the Terms of Reference for establishing WGs.

DISCONTOOLS

The information for Brucellosis was prepared in February 2013 and is currently being updated. 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.

The Complement Fixation test (CFT), iELISA, cELISA and a fluorescence polarisation assay (FPA) are the currently prescribed tests for international trade in cattle. The Rose Bengal test (RBT), CFT, FPA and brucellin

[12] 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.

skin tests are the prescribed tests in small ruminants (*B. melitensis* infection). RBT, iELISA, cELISA and FPA are the prescribed tests for *B. suis* in pigs. 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.

There are no commercially available PCR kits that claim to diagnose brucellosis. 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, but cheaper alternatives are in development.

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

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 is as practical and/or effective as the current vaccines. The effective vaccines are currently based on 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). 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

Pathogenesis and immunobiology of brucellosis : review of Brucella–host interactions (de Figueiredo et al., 2015[13])

This article, published in The American journal of pathology, reviewed *Brucella*–host interactions and immunobiology, discussing recent discoveries as the basis for pathogenesis-informed rationales to prevent or treat brucellosis. The intracellular lifestyle *Brucella* displays limits its exposure to innate and adaptive

[13] de Figueiredo, P., Ficht, T. A., Rice-Ficht, A., Rossetti, C. A., & Adams, L. G. (2015). Pathogenesis and immunobiology of brucellosis: review of brucella–host interactions. The American journal of pathology, 185(6), 1505-1517.

immune responses, sequesters the organism from the effects of antibiotics, and drives clinical disease manifestations and pathology. Brucellae exploit stealthy strategies to establish infection, including i) evasion of intracellular destruction by restricting fusion of type IV secretion system-dependent *Brucella*-containing vacuoles with lysosomal compartments, ii) inhibition of apoptosis of infected mononuclear cells, and iii) prevention of dendritic cell maturation, antigen presentation, and activation of naive T cells. The article illustrated data sets of next-generation sequences of *Brucella* and host time-series global expression fused with proteomics and metabolomics data from *in vitro* and *in vivo* experiments, which informed interactive cellular pathways and gene regulatory networks enabling full-scale systems biology analysis. Newly identified *Brucella* effector proteins, which may represent targets for improved and safer brucellosis vaccines and therapeutics, were described as well.

Improved immunogenicity and protective efficacy of a divalent DNA vaccine encoding Brucella L7/L12-truncated Omp31 fusion protein by a DNA priming and protein boosting regimen (Golshani et al., 2015[14])

This article, published in Molecular immunology, described the study of a new divalent DNA vaccine for *Brucella* with improved immunogenicity and protective efficacy. The outer membrane protein 31 (Omp31) and L7/L12 are immunodominant and protective antigens conserved among human *Brucella* pathogens (*B. melitensis* and *B. abortus*), and were used to develop DNA/DNA and DNA/Pro vaccine in the described study. Vaccination of BALB/c mice with the DNA/Pro regimen provided more protection levels against *B. melitensis* and *B. abortus* challenge than did the DNA/DNA regimen. IgG1 and IgG2a titres were higher in the sera from DNA/Pro-immunised mice than in those from mice immunised with DNA alone. Moreover, splenocytes from DNA/Pro-immunised mice produced significantly higher levels of IFN-γ than did those from mice given DNA alone. Lastly, the pcDNA-L7/L12-TOmp31 priming followed by rL7/L12-TOmp31 boosting led to improved protection against *B. abortus* or *B. melitensis* infection.

Novel real-time PCR detection assay for *Brucella suis* (Hänsel et al., 2015[15])

In this article, Hänsel and colleagues described the development of a novel real-time PCR detection assay for *Brucella suis*, as to fill the gap in sensitivity and specificity of the available ones. The authors used a bioinformatic approach to identify a *B. suis* specific 17 bp repeat on chromosome II, being common for *B. suis* biovars 1 to 4, which was used to develop a TaqMan probe assay. This assay demonstrated the highest sensitivity of all previously described *B. suis* specific PCR assays, making it possible to detect 3-4 bacterial

[14] Golshani, M., Rafati, S., Siadat, S. D., Nejati-Moheimani, M., Shahcheraghi, F., Arsang, A., & Bouzari, S. (2015). Improved immunogenicity and protective efficacy of a divalent DNA vaccine encoding Brucella L7/L12-truncated Omp31 fusion protein by a DNA priming and protein boosting regimen. Molecular immunology, 66(2), 384-391.
[15] Hänsel, C., Mertens, K., Elschner, M. C., & Melzer, F. (2015). Novel real-time PCR detection assay for Brucella suis. Veterinary record open, 2(1), e000084.

genomes per 1 µl of sample. The assay was tested 100% specific for *B. suis* and negative for other *Brucella spp*. and closely related non-*Brucella* species. The authors concluded that this novel qPCR assay could become a rapid, inexpensive and reliable screening method for large sample pools of *B. suis* biovars 1 to 4, and could be applicable for field samples, after validation.

A review of the basis of the immunological diagnosis of ruminant brucellosis (Ducrotoy et al., 2016[16])

A huge number of diagnostic tests for brucellosis have been developed, varying in antigen type, antigen presentation, antibody and conditions for the reaction, the response detected and the sample required. In this review, published in Veterinary immunology and immunopathology, *Brucella* antigens were examined focusing on cellular topology, supramolecular properties, epitopic structure, and lipopolysaccharide and protein cross-reactivity in the various contexts of immune response in ruminants. The authors discussed the significance of these features in diagnostic tests that use whole bacteria, with respect to the activities of ruminant immunoglobulins, and the effect of pH on unspecific agglutinations, non-agglutinating and blocking antibodies, pseudo-prozones and complement activation. Similarly, the bacterial surface lipopolysaccharides and cognate polysaccharides are discussed concerning topological effects, epitope exposure, ionic strength and antibody avidity in immunoprecipitation, immunosorbent and fluorescence polarization assays. Finally, the search for immunodominant protein antigens and their use in immunological tests was reviewed. The authors concluded that a critical review of the existing information would be necessary both to select optimal tests according to the logistical means available and the epidemiological context, and to focus the development of new tests.

A novel recombinant multi-epitope protein against *Brucella melitensis* infection (Yin et al., 2016[17])

In this paper, Yin and colleagues described the development of a novel recombinant multi-epitope antigen for brucellosis vaccination against *B. melitensis*, which might represent a safer and more efficacious alternative to live *Brucella* vaccines. The authors employed bioinformatics tools to predict B and T cell epitopes and evaluated the protective capacity of the recombinant antigen using mouse model of brucellosis. The results indicated that BALB/c mice immunised with this recombinant multi-epitope antigen showed mixed Th1-Th2 immune responses with high levels of specific IgG and exhibited high degrees of IFN-γ and IL-6 and significantly higher CD3, CD4, and CD8 frequencies compared to the control group. The

[16] Ducrotoy, M. J., Conde-Álvarez, R., Blasco, J. M., & Moriyón, I. (2016). A review of the basis of the immunological diagnosis of ruminant brucellosis. Veterinary immunology and immunopathology, 171, 81-102.
[17] Yin, D., Li, L., Song, D., Liu, Y., Ju, W., Song, X., ... & Li, J. (2016). A novel recombinant multi-epitope protein against Brucella melitensis infection. Immunology letters, 175, 1-7.

recombinant antigen and vaccine strain M5-90 also provided protection against *B. melitensis* 16 M infection.

Comprehensive identification of immunodominant proteins of *Brucella abortus* and *Brucella melitensis* using antibodies in the sera from naturally infected hosts (Wareth et al., 2016[18])

In this article, published in the International journal of molecular sciences, the authors described the identification of *Brucella* species-specific proteins from *B. abortus* and *B. melitensis* using sera collected from naturally infected host species. The analyses revealed 402 differentially expressed proteins, among which 63 and 103 proteins were found exclusively in the whole cell extracts of *B. abortus* and *B. melitensis* field strains, respectively. The sera from four different naturally infected host species, *i.e.,* cattle, buffalo, sheep, and goat were applied to identify the immune-binding protein spots present in the whole protein extracts from the isolated *B. abortus* and *B. melitensis* field strains and resolved on two-dimensional gel electrophoresis. Comprehensive analysis revealed that 25 proteins of *B. abortus* and 20 proteins of *B. melitensis* were distinctly immunoreactive. The authors suggested that the identified proteins could be used for the design of serological assays capable of detecting pan-Brucella, *B. abortus*- and *B. melitensis*-specific antibodies.

In vitro synergistic effects of a short cationic peptide and clinically used antibiotics against drug-resistant isolates of *Brucella melitensis* (Azad et al., 2017[19])

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.

[18] Wareth, G., Eravci, M., Weise, C., Roesler, U., Melzer, F., Sprague, L. D., ... & Murugaiyan, J. (2016). Comprehensive identification of immunodominant proteins of Brucella abortus and Brucella melitensis using antibodies in the sera from naturally infected hosts. International journal of molecular sciences, 17(5), 659.

[19] 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.

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.

The next meeting will be held in Seoul (Republic of Korea) in 25-27th October 2017.

The GFRA recently published the outcomes of their latest gap analyses 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. A dedicated meeting for updating the research gap analyses should be planned, most likely, in 2018.

DISCONTOOLS

The information o FMD was last updated in May 2015. 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

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. A constraint is the lack of sufficient panels for test validation across all serotypes and species.

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 quite 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[20])

This paper is the first of a series of seven articles that appeared in Transboundary and Emerging 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[21])

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

[20] 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 emerging diseases, 63(S1), 3-13.

[21] 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.

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[22])

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[23])

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

[22] 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.
[23] 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.

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[24])

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[25])

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-neutralizing antibodies during FMDV infection and the interactions of antibody-bound virus with immune cells. This knowledge is directly relevant to vaccine

[24] 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.

[25] 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.

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.

Global foot-and-mouth disease research update and gap analysis: 7–Pathogenesis and molecular biology (Robinson et al., 2016[26])

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.

[26] 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.

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 standardization 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 two successful meetings so far and the next meeting is scheduled for 26-27th October in Toulouse (France). On these meetings, members present overviews of their current research areas and discuss pathways for collaboration or new ideas to be explored. Contributions over the past 2 years include: update of DISCONTOOLS information for nematodes and liver fluke, response to 4 international grant calls (2 Horizon 2020 – Programme Food security and sustainable agriculture, 1 Horizon 2020 Marie-Sklodowska-Curie Action and 1 COST Action). A COST Action with the aim to foster collaborative and excellent research to COMbat Anthelmintic Resistance (COMBAR, CA16230) was recently approved and the kick-off meeting took place on 19 September 2017 in Brussels. In addition, LiHRA-members wrote 2 peer reviewed articles covering the gaps in research on the control of gastrointestinal nematodes and liver fluke in livestock, which will appear in the journal Transboundary and Emerging Diseases.

DISCONTOOLS

The database was last updated in 2013 for liver fluke and 2015 for nematodes. A new update for liver fluke is in progress and expected to appear by the end of 2017. 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.

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. Elimination of worms observed by farmer reassures him/her of necessity to treat.

The conventional diagnosis of nematode infections is laborious and expensive, and often not informative in providing a decision on whether to treat or not. 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 have a market place as monovalent vaccines. However, the ambition should be 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, and these detect evidence of exposure not necessarily current infection. Little information is available about how quickly antibody levels drop in response to treatment or loss of infection due to self-cure. Bulk tank tests are available for dairy cattle. There is a need for i) pen-side tests, ii) herd level tests to identify heavily infected herds, iii) tests for diagnosis especially for acute infection in sheep and iv) for the rapid diagnosis of new and recent infections.
Copro-antigen detection ELISAs are commercially available and can be used in any host species but these have not been fully evaluated in the field. Faecal egg count kits are available but, whilst faecal egg counts are a useful indicator of infection, these need validation for composite samples. 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 disease. DIVA based vaccines are also important to reduce unnecessary treatment hence diagnostic tests to discriminate between vaccinated animals and naturally infected animals are required. There is also a need to develop new delivery and adjuvant systems to improve level of protection and to understand the level of protection required to be commercially viable.

Pharmaceuticals

The prophylactic use of anthelmintics is currently the main method for prevention and control of disease. There are a number of anthelmintics available, triclabendazole being the anthelmintic of choice because of its

proven efficacy against young immature stages of *Fasciola*. Other than triclabendazole, there are no fully effective drugs against the young juvenile stages of the parasite, which are highly pathogenic.

Resistance in fluke populations to triclabendazole has been reported in many countries (Australia, Europe and S. America) with a need for more information about prevalence of resistance to triclabendazole. Research into strategic treatment regimens 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 extent of drug resistance.

Recent developments

Exploring the gastrointestinal "nemabiome": deep amplicon sequencing to quantify the species composition of parasitic nematode communities (Avramenko et al., 2015[27])

In this article published in Plos One, the authors describe the first application of deep amplicon sequencing to study parasitic nematode communities as well as to introduce the concept of the gastro-intestinal "nemabiome". The approach is analogous to 16S rDNA deep sequencing used to explore microbial communities, but uses the nematode ITS-2 rDNA locus instead. Gastro-intestinal parasites of cattle were used to develop the concept. They demonstrated that accurate relative quantitation of gastro-intestinal parasitic nematode communities in cattle faecal samples can be achieved. The results illustrated the insights that can be gained into the species composition of parasite communities, using grazing cattle in the mid-west USA as an example. Both the technical approach and the concept of the 'nemabiome' have a wide range of potential applications in human and veterinary medicine. These include investigations of host-parasite and parasite-parasite interactions during co-infection, parasite epidemiology, parasite ecology and the response of parasite populations to both drug treatments and control programmes.

ECONOHEALTH: Placing helminth infections of livestock in an economic and social context (Charlier et al., 2015[28])

Livestock farming is central to global food security and to the sustainability of rural communities. In this conceptual paper published in Veterinary Parasitology, the authors investigated how the inclusion of economic and social sciences can increase our understanding of the factors that drive animal health. The concept is elaborated using the example of the major helminthic diseases of cattle. They advocated (1) the

[27] Avramenko, R. W., Redman, E. M., Lewis, R., Yazwinski, T. A., Wasmuth, J. D., & Gilleard, J. S. (2015). Exploring the Gastrointestinal "Nemabiome": Deep Amplicon Sequencing to Quantify the Species Composition of Parasitic Nematode Communities. *Plos One, 10*(12).

[28] Charlier, J., Velde, F. V., van der Voort, M., Van Meensel, J., Lauwers, L., Cauberghe, V., . . . Claerebout, E. (2015). ECONOHEALTH: Placing helminth infections of livestock in an economic and social context. *Veterinary Parasitology, 212*(1-2), 62-67.

use of efficiency analysis to link animal disease with a farm's input (e.g. feed, animal health costs, labour) allocation and (2) the use of socio-psychological models to incorporate intrinsic motivations on farmers' decision making processes, whilst still considering the extrinsic (*i.e*. rational, technical and economic) factors. This should generate more integrated, situation-adapted and thus more effective prevention strategies against production diseases of animals in general, and helminth disease of ruminants in particular.

Evidence for reversion towards anthelmintic susceptibility in *Teladorsagia circumcincta* in response to resistance management programmes (Leathwick et al., 2015[29])

Maintaining production and economic viability in the face of resistance to multiple anthelmintic actives is a challenge for farmers in many countries. In this situation, most farmers in New Zealand rely on the use of combination products, containing multiple actives with similar spectra of activity, in order to maintain control. However, there are concerns that use of combinations, once resistance has already developed to the individual actives, could rapidly lead to complete failure of all actives. This study, published in International Journal for Parasitology – Drugs & Drug resistance, followed seven farms, previously diagnosed with resistance to at least two classes of anthelmintic, which were implementing a tailored programme of 'best practice parasite management'. The aim was to ascertain whether the programmes, which included the almost exclusive use of combination anthelmintics, were able to prevent resistance from developing further. Annual faecal egg count reduction tests (FECRT) were undertaken in lambs on all farms to monitor anthelmintic efficacy over 5 years. The efficacy of albendazole, ivermectin and levamisole was calculated and the changes in efficacy against *Teladorsagia circumcincta* assessed. Overall, there was a significant improvement in the effectiveness of both levamisole and ivermectin against *T. circumcincta*, and a positive but non-significant trend in efficacy of albendazole, *i.e*. there was evidence for reversion towards susceptibility. Hence, the authors concluded that the almost exclusive use of combination anthelmintics, integrated with other resistance management strategies, did not result in further resistance development despite all farms exhibiting resistance to multiple actives at the outset. What-is-more, the measured increases in anthelmintic efficacy suggests that adoption of best practice management strategies may extend the useful life of anthelmintics even after resistance has been diagnosed.

[29] Leathwick, D. M., Ganesh, S., & Waghorn, T. S. (2015). Evidence for reversion towards anthelmintic susceptibility in Teladorsagia circumcincta in response to resistance management programmes. *International Journal for Parasitology-Drugs and Drug Resistance, 5*(1), 9-15.

Serological examination of fattening pigs reveals associations between *Ascaris suum*, lung pathogens and technical performance parameters (Vlaminck et al., 2015[30])

Currently, the only available routine measure to assess exposure of fattening pigs to *Ascaris suum* is the inspection of livers at slaughter. In this paper, published in Veterinary Parasitology, Vlaminck et al. reported a new serological test, based on the detection of antibodies to the *A. suum* haemoglobin molecule. The test showed to be highly sensitive for the detection of exposure to *A. suum* in fattening pigs. In addition, a significant relationship was detected between elevated average *Ascaris* serology and percentages of affected livers. Ascaris serology and the percentage of affected livers were negatively correlated with average daily gain (ADG). In some farms, correlations between the percentage of affected lungs at slaughter and elevated presence of *A. suum* and several other airway pathogens were detected. The authors concluded that serological screening for *A. suum* on fattening farms is an attractive new diagnostic tool that can be used to indicate the presence of roundworm infection by measuring infection intensity.

Modelling the consequences of targeted selective treatment strategies on performance and emergence of anthelmintic resistance amongst grazing calves (Berk et al., 2016[31])

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,

[30] Vlaminck, J., Dusseldorf, S., Heres, L., & Geldhof, P. (2015). Serological examination of fattening pigs reveals associations between *Ascaris suum*, lung pathogens and technical performance parameters. *Veterinary Parasitology, 210*(3-4), 151-158.

[31] 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.

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.

Climate-driven longitudinal trends in pasture-borne helminth infections of dairy cattle (Charlier et al., 2016[32])

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[33])

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 multicellular parasite vaccine to the point of practical application would be a groundbreaking step in the control of these important endemic infections of livestock.

[32] 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.

[33] 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.

Automated parasite faecal egg counting using fluorescence labelling, smartphone image capture and computational image analysis (Slusarewicz et al., 2016[34])

In a paper published in the International Journal for Parasitology, the authors reported the use of chitin as a potential universal marker of nematode eggs. They constructed a smartphone-based system for parasite faecal egg counts and the generated egg counts had good agreement with the results obtained from a traditional faecal egg counting method. Coefficients of variation for smartphone counts were significantly lower and the system was capable of differentiating ascarid and strongyle eggs. The results demonstrated the feasibility of a simple, automated on-site test to detect and/or enumerate parasite eggs in mammalian faeces without the need for a laboratory microscope, and highlighted the potential of smartphones as relatively sophisticated, inexpensive and portable medical diagnostic devices.

Comparison between anthelmintic treatment strategies against *Ascaridia galli* in commercial laying hens (Tarbiat et al., 2016[35])

In a study published in Veterinary Parasitology, the efficacy of a sustainable deworming strategy based on targeted treatments (TT) against *Ascaridia galli* was investigated. Three experimental protocols with different levels of treatment, *e.g*. targeted treatment (TT), conventional treatment (CT) and untreated (UT), were tested in randomly allocated flocks of two different bird hybrids. Every second week, faecal egg counts (FECs) were determined from pooled faecal materials. In the TT, anthelmintic administration (fenbendazole, 1 mg/kg body weight for 5 days) started at 22 weeks post placement (wpp) and was repeated twice when the pooled FECs surpassed the assigned threshold of 200 egg per gram faeces (EPG). The CT flocks were treated once at 27 wpp using the same anthelmintic. Hens in the UT were not dewormed and served as controls. None of the flocks became infected until after 16 wpp. The cumulative pooled FECs at the end of the study were significantly ($p < 0.01$) lower in the TT compared to both CT and UT. Cloacal FECs and the number of adult *A. galli* in TT at 35 and 45 wpp were significantly lower compared to other flocks. The TT strategy was better in terms of lower worm burden and decreased cumulative environmental parasite egg numbers compared to the CT strategy. The authors concluded that the TT strategy should be considered as an alternative to the CT strategy with regard to *A. galli* control in commercial laying hens.

[34] Slusarewicz, P., Pagano, S., Mills, C., Popa, G., Chow, K. M., Mendenhall, M., . . . Nielsen, M. K. (2016). Automated parasite faecal egg counting using fluorescence labelling, smartphone image capture and computational image analysis. *International Journal for Parasitology, 46*(8), 485-493.

[35] Tarbiat, B., Jansson, D. S., Tyden, E., & Höglund, J. (2016). Comparison between anthelmintic treatment strategies against *Ascaridia galli* in commercial laying hens. *Veterinary Parasitology, 226*, 109-115.

Utilization of composite fecal samples for detection of anthelmintic resistance in gastrointestinal nematodes of cattle (George et al., 2017[36])

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[37])

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 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.

[36] 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.

[37] 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

Multiplexed-tandem PCR for the specific diagnosis of gastrointestinal nematode infections in sheep: an European validation study (Roeber et al., 2017[38])

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.

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 group has since been established, but the IRC Secretariat is currently working to expand the initial list of experts so as to establish a STAR-IDAZ IRC Working Group (WG), following the procedures indicated in the Terms of Reference for establishing WGs.

DISCONTOOLS

The information on PRRS was prepared in September 2009. An update of the information is in progress. 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. The indirect immunoperoxidase monolayer assay (IPMA) and indirect immunofluorescence use alveolar macrophages and MARC-145 cells. A wide variety of ELISAs has been developed with some identifying both the US and European strains whilst others can differentiate strains. Diagnostic kits (PCRs, ELISAs) are available worldwide but it is questionable whether they can pick up all circulating isolates, especially with the

[38] 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*.

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 with the existing PCRs/ELISAs. To achieve this requires regular pathological studies for new virus strains, isolation of the virus, whole genome sequencing and antisera production. This is also essential to study the evolution of the virus and its rapid genetic changes.

Vaccines

Both attenuated live and inactivated vaccines are available, containing either USA or European viruses. Inactivated vaccines area safe but not efficacious. They can only boost the existing immune response in sows. Inactivated vaccines do not protect naïve animals and give only boost reactions when the circulating virus resembles closely the vaccine virus. Attenuated vaccine viruses are not fully safe as horizontal and vertical spread, shedding in semen and reversion to virulence may occur. Attenuated vaccines were very effective as long as the circulating strains were closely related to the vaccine strain. With the genetic drift of the circulating PRRS virus, vaccines lose their efficiency. At present, strains exist that easily escape from the protection induced by vaccines.

Vaccines should match the naturally occurring endemic strains and consequently there is an urgent need for vaccines adapted to the circulating strains. To achieve this, development of multivalent vaccines should be considered. Commercial companies in the field are interested but there are no vaccines against the more recent strains or multiple strains. There is an urgent need for an update of the existing vaccines.

Pharmaceuticals

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

Recent developments

PRRSV structure, replication and recombination: origin of phenotype and genotype diversity (Kappes and Faaberg, 2015[39])

An article published on Virology reviewed the research on virion structure, replication and recombination properties of PRRSV that have led to the extraordinary phenotype and genotype diversity that exists worldwide. The authors discussed structure, replication and recombination mechanisms that have yielded genotypic and phenotypic diversity. This background information would be of use for understanding the reasons as to why this pathogen has continued to frustrate efforts to eliminate infection of herds through vaccination or other elimination strategies.

[39] Kappes, M. A., & Faaberg, K. S. (2015). PRRSV structure, replication and recombination: origin of phenotype and genotype diversity. Virology, 479, 475-486.

PRRS virus receptors and their role for pathogenesis (Zhang and Yoo, 2015[40])

In their article, published on Veterinary Microbiology, Zhang and Yoo discussed PRRS virus (PRRSV) receptors and their role for pathogenesis, describing the advances and current understanding of the entry of PRRSV to cells with a particular focus on the role of CD163 and sialoadhesin in relation to infection and PRRSV pathogenesis. At least six cellular molecules have been described so far as putative receptors for PRRSV. CD163 is most likely the primary and core receptor for PRRSV and determines the susceptibility of cells to the virus. Sialoadhesin is either unnecessary for infection or may function as an accessory protein. The authors highlighted two main knowledge gaps on this topic. First, sialoadhesin has been mostly studied for genotype I PRRSV whereas the utilisation of CD163 has been mostly studied using genotype II PRRSV, and whether each genotype utilises a different receptor is unclear. Secondly, PRRSV-associated membrane fusion has not been documented yet, and thus the molecular basis for the release of viral nucleocapsid from the endosomal cavity to the cytoplasm remains to be determined.

Porcine reproductive and respiratory syndrome virus (PRRSV): pathogenesis and interaction with the immune system (Lunney et al., 2016[41])

In this review, published in Annual review of animal biosciences, Lunney and colleagues addressed important issues of PRRSV infection, immunity, pathogenesis, and control, with the major goal of identifying cellular/viral targets and pathways for designing more effective vaccines and therapeutics. The paper discussed latest information on viral genome structure, pathogenic mechanisms, and host immunity, with a special focus on immune factors that modulate PRRSV infections during the acute and chronic/persistent disease phases. In addition, the authors addressed genetic control of host resistance and probe effects of PRRSV infection on reproductive traits. Based on progress in viral reverse genetics, host transcriptomics and genomics, and vaccinology and adjuvant technologies, the paper identified new areas for PRRS control and prevention, and highlighted the knowledge gaps and the need for advanced molecular and immune tools to stimulate PRRS research and field applications.

Gene-edited pigs are protected from porcine reproductive and respiratory syndrome virus (Whitworth et al., 2016[42])

An article published in Nature Biotechnology suggested that the inactivation of the pig gene CD163, which is the receptor for entry of PRRSV into cells, would provide immunity to PRRSV infection in pigs. The

[40] Zhang, Q., & Yoo, D. (2015). PRRS virus receptors and their role for pathogenesis. Veterinary microbiology, 177(3), 229-241.
[41] Lunney, J. K., Fang, Y., Ladinig, A., Chen, N., Li, Y., Rowland, B., & Renukaradhya, G. J. (2016). Porcine reproductive and respiratory syndrome virus (PRRSV): pathogenesis and interaction with the immune system. Annual review of animal biosciences, 4, 129-154.
[42] Whitworth, K. M., Rowland, R. R., Ewen, C. L., Trible, B. R., Kerrigan, M. A., Cino-Ozuna, A. G., ... & Wells, K. D. (2016). Gene-edited pigs are protected from porcine reproductive and respiratory syndrome virus. Nature Biotechnology, 34(1), 20-22.

authors generated gene edited pigs lacking CD163, demonstrating that the animals were resistant to challenge with a relatively virulent viral isolate. These results would pave the way for vaccine development as well as for genetic selection programmes. The authors concluded that the use of such genome-edited animals in agriculture could substantially reduce PRRS-related economic losses.

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[43])

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[44])

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.

[43] 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.

[44] 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.

Antiviral Strategies against PRRSV Infection (Du et al., in press[45])

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 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.

[45] Antiviral Strategies against PRRSV Infection. Trends in Microbiology.