

# Global Foot-and-Mouth Disease Research Update and Gap Analysis 2014



Compiled and written by  
Drs Lucy Robinson and Theo Knight-Jones

## The Global Foot-and-Mouth Disease Research Alliance

The Global Foot-and-Mouth Disease Research Alliance (GFRA) comprises 16 international institutions actively engaged in Foot-and-Mouth Disease (FMD) research, as well as numerous partners, collaborators and stakeholders. The GFRA has five strategic goals aimed at expanding FMD research collaborations and optimizing use of resources and expertise:

- **Goal 1.** To facilitate research collaborations and serve as a communication gateway for the global FMD research community.
- **Goal 2.** To conduct strategic research to better understand FMD.
- **Goal 3.** To develop the next generation of control measures and strategies for their application.
- **Goal 4.** To determine social and economic impacts of the new generation of improved FMD control measures.
- **Goal 5.** To provide evidence to inform development of policies for safe trade of animals and animal products in FMD-endemic areas.

For more information, including current GFRA news and members, see <http://www.ars.usda.gov/gfra/>

## Purpose of the report

There are numerous FMD research organisations and experts. The broad focus of their research is driven by funding bodies, which in turn are directed by scientists, policy makers and non-governmental donors. It is important that each of these groups is aware of the current needs and gaps in FMD knowledge, technologies and research capacity. The purpose of this document is to provide appropriate information in these areas to facilitate an efficient and coordinated approach to global FMD research.

Here, we provide a fully-referenced update of the research conducted on FMD and the FMD virus (FMDV) since the previous GFRA report in 2011. Alongside a review of the literature pertaining to priority research areas, current activities being undertaken at GFRA and EuFMD (European Commission for the Control of FMD) member institutions are included, alongside an analysis of the major gaps in knowledge and a summary of proposed next-steps.

This information is presented in the context of the GFRA goals, with reference to the parent report, the 2010 Gap Analysis produced by The FMD Countermeasures Working Group in Buenos Aires, Argentina. The Gap Analysis provided a comprehensive summary of the priority research areas according to the state of knowledge in 2010, and therefore serves as a useful reference for the progress made in recent years. As well as a research update, it is our hope that this document will enable researchers to effectively target their work towards prioritized knowledge gaps, avoid duplication of efforts, and highlight opportunities for collaboration.

A list of contributing institutes is provided in the Appendices.

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## Executive Summary

In this report we combine literature review and expert consultation to evaluate global FMD research progress since 2011 and highlight remaining knowledge gaps, incorporating ongoing work from 33 research institutes from around the world.

### *Global needs:*

FMD is frequently listed as the most economically important disease of livestock in both developed and developing countries. Disease-free countries experience periodic FMD outbreaks and so must maintain their capacity for rapid detection and control. Some countries, particularly in South America, are successfully controlling and eradicating FMD through mass vaccination. However challenges remain in proving FMDV freedom in vaccinated populations and in the design, production and distribution of vaccines able to induce effective immunity to emergent field strains. In addition, many other endemic countries have no, or ineffective, control policies or programmes. In these cases there is an urgent need to understand the economic and social impacts of FMD within each region, and to determine whether or how FMD can be controlled by vaccination alone. Underpinning all this is the requirement for comprehensive knowledge of the virus itself, its interaction with host species, and how we can most effectively prevent its replication and spread within both individual animals and through populations.

It is commonly accepted that FMD vaccines are not very stable and this has been historically blamed for their low levels of protection from FMDV in the field. While this principle is true, it has been highlighted in recent years that the manufacturing process itself warrants significant scrutiny moving forwards. A strong regulatory framework surrounding vaccine production is necessary to ensure antigen stability and to assure that maximum potency is realised under field conditions. These findings are discussed in more detail within the Vaccines section of this report.

### *Recent developments:*

Since the last GFRA report, the USA has licensed a recombinant vector vaccine for use in cattle during an outbreak of FMD. Additional new vaccine technologies that address important gaps have also been transferred from GFRA research laboratories to vaccine manufacturers and are currently under development: a program of rolling out these new technologies to other parts of the world is likely to prove valuable in years to come. Studies of the interaction of the virus with the immune systems of various hosts are informing promising rational improvements to vaccine design and testing. Coupled with advances in manufacturing and quality control, we can be hopeful of improved outcomes from established vaccination programs in the medium term future.

In terms of molecular analysis, advanced genetic studies have improved understanding of FMD transmission with increasing relevance to field control. Development and scrutiny of mathematical models has enhanced outbreak preparedness and policy planning, although more ground testing is required. Novel molecular techniques promise to further reduce diagnostic costs and improve accessibility to high quality testing.

Greater consideration is being given to commodity-based trade as a way of opening up FMD-free trade to poor farmers in wildlife endemic areas without the economic drain and ecological impact of zonation and enclosure of wildlife.

Previously neglected, the importance of field epidemiology in the evaluation of FMD outbreaks and control programmes is being increasingly recognised.

## **Research priorities by area:**

### ***Controlling the disease***

#### **Epidemiology**

Although the field of FMD modelling is progressing, validated best practices have not yet been established: rigorous ground testing of real time decision support tools, including those capable of estimating and comparing cost-effectiveness of different strategies will be required. The extent to which generic spread models can be used in different settings also needs to be determined.

Further developments are required in defining appropriate surveillance methods, particularly those able to confirm of disease-free status in vaccinated populations.

Clear guidance, knowledge sharing and evidence-based policy developments will be necessary to assist endemic countries to control FMD, with a focus on those possessing substantial smallholder farming sectors. At the same time, basic field epidemiology has been under-utilised in the evaluation of control programmes, particularly in endemic countries. A pressing question in this area is to what extent we can hope to control FMD by vaccination in the absence of effective biosecurity measures, which are notoriously difficult to enforce in some settings.

The use of molecular and genetic techniques in combination with epidemiology is expanding as technologies become cheaper and more powerful. This progress must be supported, as major

breakthroughs in our ability to understand and ultimately control FMD could be imminent. The incorporation of these tools into practical disease control should also be encouraged. However, the ability to use models to predict severity and likelihood of transmission based on genetic data remains elusive.

In the case of FMD-free countries, approaches for identifying high risk strains for incursion and estimating their impact should be developed. Efforts to maintain outbreak preparedness, including training, planning of control strategy and resource requirements need to be continued.

### **Wildlife**

The need for improved understanding of the role of wildlife in FMD maintenance and transmission is evident. Characterising the features of infection and transmission in controlled studies and field studies of relevant species will be required to provide accurate information to inform policy on the role of wildlife in official FMD status.

### **Socio-economics and international standards**

The full social and economic impact of FMD remains hard to estimate, which hampers effective design of control policies. In particular, in endemic regions with limited potential to access export markets, we currently know little of the impact of FMD and its control measures. High quality information would assist evaluation of FMD control options in order to mitigate disease impact.

Social factors profoundly affect the effectiveness of monitoring and control policies. In particular we need to understand how best to use compensation as a means of maximising farmer participation in disease reporting and control. Furthermore, a wider appreciation of the regional social and cultural factors that affect compliance would be beneficial.

Real-time economic models to guide disease control during outbreaks have been developed and must now be rigorously field tested.

### **Vaccines**

The use of reverse genetics to engineer new FMD vaccines, such as inactivated leaderless FMD vaccines, viruses with improved stability and culture adaptation, and FMD virus-like particles, has the potential to revolutionise the safe and cost-effective production of effective FMDV vaccines. However, careful evaluation of novel vaccines is needed with a particular focus on their efficacy in species other than cattle and their effects on non-humoral aspects of the immune response.

Several novel adjuvanting or vectoring strategies for various FMD vaccines also show promise but will require further testing in a range of species under physiological challenge conditions. Whilst convenient and cheap, the use of murine systems should be explored with extreme caution in light of accumulating data on relevant differences between their immune systems and those of natural FMD target species. In addition, the importance of qualitative aspects of the response to FMDV infection and vaccination, such as the characteristics of non-neutralising antibodies, or the immunoglobulin isotype ratio induced, must be recognized, and these parameters should be included during the evaluation of novel adjuvants or vaccines. To provide meaningful reference, conventional vaccines should be included alongside any novel approach in all studies.

Improved methods enabling the rapid and effective purification of FMDV for conventional vaccine production are under development which, if rolled out widely, could improve control in endemic areas within a relatively short time.

Some studies have begun to indicate possible ways of using needle-free strategies to induce protective mucosal responses to FMDV vaccines, but further study of mucosal immunity in target species (perhaps requiring the development of additional tools), both in general and in relation to FMD, will be required to realise the potential of this strategy.

Predicting vaccine matching with emerging field strains remains challenging.

The control of FMD in endemic regions would be markedly assisted by improving the formulation of quality controlled vaccines and reagents to extend shelf life and reduce cost, thereby potentially increasing both availability and efficacy. More broadly, standardised independent quality assurance should be used by all FMD vaccine production facilities.

Despite much effort, reliable immune correlates of protection remain to be identified. An absence of standardised approaches to vaccination and challenge during early vaccine development is likely to have contributed to this problem, and should be addressed as a matter of urgency, ideally in collaboration with the OIE and other international bodies to ensure widespread uptake of resulting recommendations. The definition of a common protocol and minimum set of basic standards for such experiments would have dramatic impacts on our ability to compare novel vaccination strategies carried out at different sites, and provide a way of understanding which approaches warrant further study and which should be discarded. The current haphazard use of different protocols under diverse conditions with individually selected readouts risks sub-optimal use of time and resources and must be considered a high priority for change.

## **Biotherapeutics**

In 2010, the use of adenovirus to vector interferon (IFN)  $\alpha$  (Ad5-IFN $\alpha$ ) into hosts and induce short-lived non-specific protection against FMDV appeared promising. It now seems that Ad5-IFN $\alpha$  alone is insufficiently potent for use in outbreak situations. However, studies combining Ad5-IFN $\alpha$  with an additional immune stimulator show promise, as does Ad5 encoding an IFN-stimulating transcription factor in place of IFN $\alpha$ . Substituting Type III IFN into the Ad5 vector appears more effective, particularly in cattle, and warrants further investigation.

An area of urgent unmet research need is the potential interaction of immune-modulating biotherapeutics with the response to vaccination. Before use in the field, we must know how expression of large amounts of these innate immune cytokines affects both qualitative and quantitative aspects of the immune response in target species. For example, high levels of IFN $\alpha$  can adversely impact the magnitude of T cell responses, which in the case of FMD vaccination, could preclude the formation of sufficient immunity to confer protection from disease. This has so far been almost completely neglected in the literature. More generally, the integration of biotherapeutics into existing emergency measures similarly remains to be achieved.

Non immunological, small molecule inhibitors of FMDV 3Cpro have been identified, but remain to be tested in target species *in vivo*.

RNA interference-based strategies are also being explored, though their suitability for upscaling to the levels required in outbreak situations has yet to be assessed.

## **Disinfectants**

While published literature has focused on the environmental impact of existing disinfectant use in recent outbreaks, ongoing work, particularly at the Plum Island Animal Disease Center (PIADC), aims to develop standardised disinfectant efficacy test methods, to assess activity of antimicrobial pesticides against FMDV, and to define optimal conditions of use for new and established disinfectants.

## **Diagnostics**

Continued development of molecular and genetic technologies combined with novel analytical techniques will benefit many areas of FMD research and understanding. Production of effective pen-side tests and increasing the usability and affordability of various FMD diagnostics for laboratories with limited resources would offer significant benefits to endemic regions in particular.



Improved diagnostics for SAT strains are still required, as are better tests to support DIVA (Differentiation of Infected and Vaccinated Animals) and for vaccine matching. Investigation of air sampling technologies is ongoing.

Further development, validation and standardisation of methods for measuring post-vaccination population immunity are required.

## ***Understanding the virus***

### **Pathogenesis**

Our understanding of FMDV persistence has been revolutionised by finding that retention of virus in the lymph node appears to be more the rule than the exception, even in species not thought capable of retaining live virus, such as swine. What remains to be seen is whether this phenomenon is primarily to the advantage of host or pathogen.

Understanding early events during infection has been hampered historically by the common use of non-physiological routes of challenge with the virus. Data now show that widespread adoption of more relevant routes of infection for laboratory studies is both feasible and desirable. Standardisation and universal acceptance of this approach would be enormously beneficial.

Beyond expression of integrins, evidence of other determinants of susceptibility at the cellular level have gained interest in recent years and should be pursued.

Factors underpinning genetic resistance and age-related susceptibility to FMDV infection warrant investigation.

Determinants of the extent of the role of wildlife species in field conditions during outbreaks should be defined with the support of laboratory controlled infections to enable full understanding of the disease in epidemiologically relevant non-agricultural species.

### **Immunology**

New knowledge on the bovine immunoglobulin response requires interpretation for its relevance to FMDV infection and vaccination in natural FMDV target species.

The importance of non-neutralizing antibodies in the response to FMDV has become apparent, particularly in terms of interactions of antibody-bound virus with immune cells. We now need to define the roles of these immune complexes *in vivo* in target species to enable their exploitation.

Regulatory gamma-delta T cells appear to play a previously unappreciated role in the response to vaccination of cattle. Comparable experiments should be undertaken in other target species to understand the impact of this cell type to FMD vaccination in broader terms, particularly in light of new data highlighting the importance of T cell responses for optimal vaccine-induced immunity. Similarly, novel approaches have revealed that CD8 T cell stimulation may also be a desirable outcome of vaccination, but its potential remains unexplored outside of swine.

The neonatal immune systems of FMDV target species remain far less well characterised than their adult counterparts. Further study of neonatal immunity, with particular reference to the T cell compartment, and in conjunction with characterisation of FMDV vaccine and adjuvant responses is required.

Qualitative hallmarks of effective adaptive immunity to FMD vaccines and infection are being uncovered. Understanding how to use this knowledge in a more comprehensive approach to vaccine design could prove valuable. Further study to determine how to achieve optimal IgG class ratios and increase immunoglobulin avidity should inform development of improved vaccines, particularly where cross-protection is a priority.

Mucosal immune responses remain relatively under-studied in target species. Robust techniques should be developed and applied to understand how mucosal and systemic immunity combine to provide effective protection following vaccination and during infection.

### **Molecular biology**

The multiple and varied ways in which FMDV manipulates the host immune system are becoming evident from molecular studies that should serve to both validate and direct design of novel biotherapeutics.

Substantial data indicate that FMDV 3A warrants further investigation as a determinant of both virulence and host tropism, but an emphasis on experimentation in natural host species is required to move forwards.

Exploitation of precise, quantitative, high-throughput molecular techniques to study FMDV holds great promise for advances in understanding of pathogenesis at the cellular level. Their future application to primary cells or more physiologically-relevant cell lines, combined with careful experimental design, will be required for this promise to be realised.

Considering recent advances in our molecular understanding of FMDV's interactions with the host interferon response, we now need to establish to what extent we can link molecular findings with whole organism responses to natural infection. As a starting point, some of the experiments that have been carried out in immortalised cell lines should be repeated in cell lines and/or primary cells from relevant target tissues of natural host species.

### **New research tools and approaches**

Large animal experimentation for FMDV is necessary, but coupling this approach with appropriate *in vitro* and small animal models of the disease has the potential to expedite research progress and reduce the number of large animals needed. However, the challenge of developing and validating the "right" model remains. In parallel, reagent/method development to enable in-depth study of the immune systems of target species, particularly the mucosal compartment, would facilitate advances in FMDV pathogenesis and vaccine science.

The connection of internationally-recognised centres of expertise in FMD with local research institutes in affected regions will enable effective transfer of knowledge and expertise to smaller centres and ensure a standardized approach to the characterisation of new isolates in common or locally-relevant host species. Some institutes are pioneering this approach, which has the potential for widespread benefit to the FMD research community. Opportunities to extend and replicate this model should be sought and supported.

## About the authors

Dr Lucy Robinson graduated with a D.Phil. in viral immunology and pathology from Oxford University in 2008, after working on FMDV in cattle at The Pirbright Institute. She completed several years of post-doctoral study both in the UK and in SE Asia, before embarking on a career in manuscript editing and scientific writing. She now runs her own consultancy, Insight Editing London ([www.insighteditinglondon.com](http://www.insighteditinglondon.com)), where she specialises in report writing and pre-submission review of biological and biomedical science manuscripts.

Dr Theo Knight-Jones started as a veterinary clinician, before specialising in veterinary epidemiology and public health. Having spent several years gaining experience and qualifications at the Royal Veterinary College, London, Theo obtained his PhD at The Pirbright Institute looking at the evaluation of FMD vaccines and vaccination programmes. He is currently based in Zambia employed as a veterinary epidemiologist at The International Livestock Research Institute (ILRI) within the Food Safety & Zoonoses group.

## Contributors

The authors are extremely grateful for the significant contributions of Bryan Charleston and Wilna Vosloo as well as the inputs of other members of the GFRA executive committee, including Cyril Gay and Luis Rodriguez. Thanks also go to Keith Sumption and members of the EuFMD Special Committee for Research and Programme Development (SCRPD) for their inputs and financial support to compile this report.

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## Abbreviations

AHL-NZ: Animal Health Laboratory – New Zealand

AHAW: Animal Health and Welfare panel (of the European Union)

ANSES: Agence Nationale de Sécurité Sanitaire de l'alimentation, de l'Environnement et du travail (National Agency for Food, Environmental and Occupational Health Safety), France

APHIS: Animal and Plant Health Service, USDA, United States of America

ARS: Agricultural Research Service, USDA, United States of America

BVI: Botswana Vaccine Institute, Botswana

CAAS: Chinese Academy of Agricultural Sciences

CD: Cluster of Differentiation

cDNA: Complimentary Deoxyribonucleic Acid (DNA)

CEVAN: Centro de Virología Animal (Center of Animal Virology), Argentina

CIRAD: Centre de Coopération Internationale en Recherche Agronomique pour le Développement (French Agricultural Research Centre for International Development), France

CODA: Centrum voor Onderzoek in Diergeneeskunde en Agrochemie (Veterinary and Agrochemical Research Center), Belgium

COX-2: Cyclo-Oxygenase 2

CSIRO-AAHL: Commonwealth Scientific and Industrial Research Organisation, Australian Animal Health Laboratory, Australia

DC: Dendritic Cell(s)

DIVA: Differentiation of/Differentiating Infected from Vaccinated Animals

DHS: Department of Homeland Security, United States of America

DNA: Deoxyribonucleic Acid

DTU: Danmarks Tekniske Universitet (Technical University of Denmark), Denmark

EFSA: European Food Safety Authority

ELISA: Enzyme-Linked Immuno-Sorbent Assay

EU: European Union

EuFMD: European Commission for the Control of FMD

FLI: Friedrich-Loeffler-Institute, Germany

FMD: Foot-and-Mouth Disease

FMD-DISCONVAC: Development, Enhancement and Complementation of Animal-sparing, FMD Vaccine-Based Control Strategies for Free and Endemic Regions

FMDV: Foot-and-Mouth Disease Virus

GFP: Green Fluorescent Protein

GFRA: Global Foot-and-Mouth Disease Research Alliance  
ICT-Milstein: Instituto de Ciencia y Tecnología Dr. César Milstein  
IFITM3: Interferon-Induced Transmembrane Protein 3  
IFN: Interferon  
Ig: Immunoglobulin  
IKK: I  $\kappa$ B Kinase  
IL: Interleukin  
ILRI: International Livestock Research Institute, Kenya  
INTA: Instituto Nacional de Tecnología Agropecuaria (National Institute of Agricultural Technology), Argentina  
IRES: Internal Ribosome Entry Site  
IVI: Institute of Virology and Immunology, Switzerland  
IVRI: Indian Veterinary Research Institute, India  
IZSLER: Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna (Lombardy and Emilia Romagna Experimental Zootechnic Institute), Italy  
LFD: Lateral Flow Device  
LPBE: Liquid Phase Blocking ELISA  
LVRI: Lanzhou Veterinary Research Institute of the Chinese Academy of Agricultural Sciences (CAAS), China  
MAb: Monoclonal Antibody  
NADDEC: National Animal Disease Diagnostics and Epidemiology Centre, Uganda  
NaLIRRI: National Livestock Resources Research Institute, Uganda  
NARC: National Agricultural Research Center, Pakistan  
NCAD: National Centers for Animal Disease, Canada  
NF- $\kappa$ B: Nuclear Factor  $\kappa$ B  
NIAB: Nuclear Institute for Agriculture and Biology, Pakistan  
NIBGE: National Institute for Biotechnology and Genetic Engineering, Pakistan  
NSP: Non-Structural Protein(s)  
NVRI: National Veterinary Research Institute, Nigeria  
ODN: Oligodeoxynucleotides  
OIE: Office International des Epizooties (World Organisation for Animal Health)  
OVI: Onderstepoort Veterinary Institute, South Africa  
PCP-FMD: Progressive Control Pathway for the control of FMD  
PCR: Polymerase Chain Reaction

PIADC: Plum Island Animal Disease Center, USA

PGE2: Prostaglandin E 2

PPRV: Peste des Petits Ruminants Virus

RNA: Ribonucleic Acid

RT-LAMP: Reverse Transcription-Loop-Mediated Isothermal Amplification

RT-PCR: Reverse Transcriptase-Polymerase Chain Reaction

SACIDS: Southern African Centre for Infectious Disease

SAT: Southern African Territories

SP: Structural Protein(s)

TLR: Toll-like Receptor(s)

USD: United States Dollars

USDA: United States Department of Agriculture

VI: Virus Isolation

VLA: Veterinary Laboratory Agency, Tanzania

VLP: Virus-Like Particle

VNT: Virus Neutralization Test

WCS-AHEAD: Wildlife Conservation Society - Animal & Human Health for the Environment And Development



## Background

In August 2010, the FMD Countermeasures Working Group was created to bring together a selection of leading authorities in the field of FMD. With the support of the GFRA and the Instituto Nacional de Tecnología Agropecuaria (INTA) of Argentina, they conducted an analysis of current knowledge of the disease and its control in both epidemic (predominantly the US) and endemic situations. At that time, the group identified several major obstacles to effective prevention, detection and control of FMD, including:

1. Poor and inadequate education and training of veterinarians and livestock producers in detecting early signs of FMD.
2. Lack of validated commercial pen-side test kits for disease control.
3. Failure of serologic methods to determine status (infected, uninfected) in some vaccinated animals.
4. Absence of a surveillance system for early recognition of signs, or to find evidence using antigen detection, antibody, or virus detection.
5. Lack of reliable comprehensive international surveillance systems to collect and analyse information.
6. Current models have not been designed to evaluate in real-time the cost-effectiveness of alternative control, surveillance, and sampling strategies.
7. Several aspects of FMD epidemiology and transmission still have to be uncovered, including the influence of viral factors that affect viral persistence, emergence, competition, transmission, and spread of FMD virus strains.
8. There are no FMD vaccines permitted for distribution and sale in the US
9. At present, there is no rapid pen-side or field-based diagnostic test for FMD control during a disease outbreak that has been validated in the field as “fit for purpose.”
10. There is a need for better analytical tools to support decisions for FMD control.

These obstacles were further broken down into priority research knowledge gaps, and in this report we will present recent progress towards filling those gaps. We will also highlight points that remain to be addressed, and identify new targets for research that have emerged as a product of improved understanding and/or changing needs since 2010.

## Report approach

This report summarises recent completed and ongoing global FMD research and thereby identifies current knowledge gaps. A literature review was conducted to identify recent published FMD research. This was based on a PubMed search using the search terms “foot and mouth” but not “hand, foot and”, published between June 2011 and June 2014. This returned a total of 505 relevant peer-reviewed manuscripts that could be approximately allocated to research topic areas as shown in Table 1.

Research Category	Papers (n)
Epidemiology	116 (23%)
Vaccines	94 (19%)
Pathogenesis	74 (15%)
Molecular biology	57 (11%)
Diagnostics	55 (11%)
Immunology	50 (10%)
Policy, preparedness and trade	38 (8%)
Wildlife	19 (4%)
Vaccine evaluation ( <i>quality, efficacy, effectiveness</i> )	16 (3%)
Other	15 (3%)
Economics	14 (3%)
Biotherapeutics	13 (3%)
<b>Total</b>	<b>505 (100%)</b>

**Table 1: FMD peer-reviewed publications June 2011-June 2014 sub-divided by topic area.**

All member institutes of the GFRA and EuFMD collaborators were also contacted and given the opportunity to contribute updates on their recent, ongoing and future FMD research activities. Responses were received from 33 institutes (see appendix - **Contributing Institutions & Financial Support**), distributed as follows:

- Europe – 10 institutes (30%)
- North America – 5 institutes (15%)
- South America – 2 institutes (6%)
- Asia – 6 institutes (18%)
- Africa – 8 institutes (24%)
- Australia/New Zealand – 2 institutes (6%)

Published literature and research updates were then summarised for each area of FMD research and evaluated in the context of the goals identified in the 2010 Gap Analysis. Any unmet goals from 2010

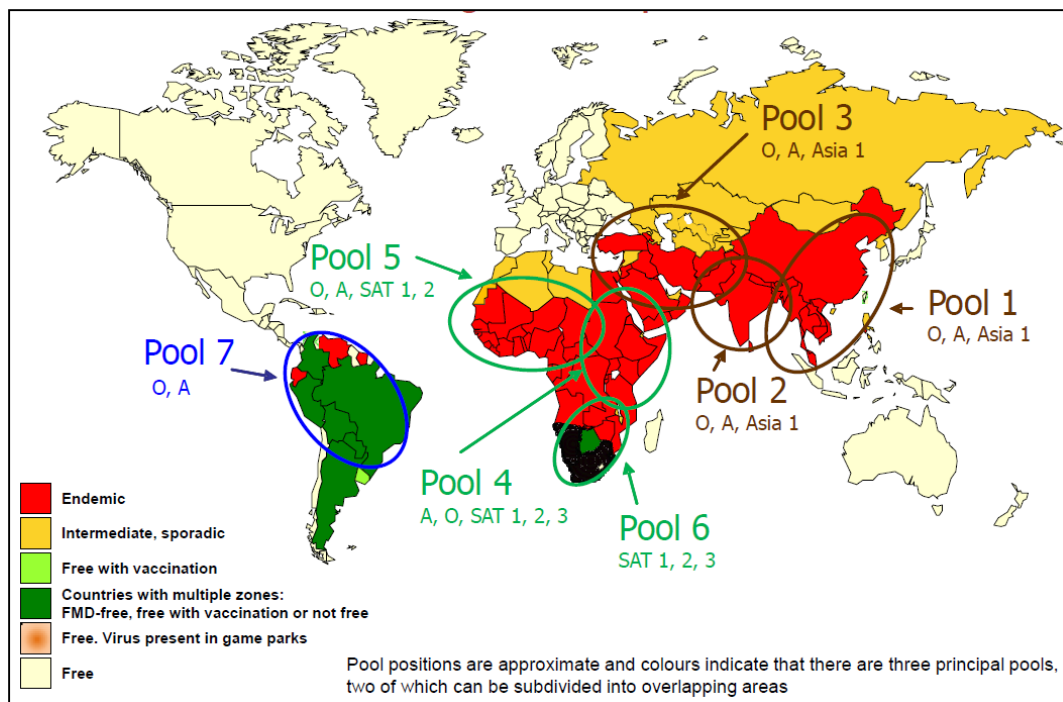
were noted and new goals were also highlighted in order to provide a comprehensive account of the most pressing topics in need of research as of June 2014.

# **Literature Review and Research Updates by Topic Area**

## Introduction

Although a disease of low overall mortality, FMD places a huge burden on both individual livestock keepers and national economies (Fogedby 1962, James and Rushton 2002, Suttmoller, Barteling et al. 2003, Knight-Jones and Rushton 2013). It is a viral disease (family: Picornaviridae, genus: Aphthovirus) affecting cloven-hoofed animals, the epidemiologically and economically most important hosts being cattle, water buffalo, pigs, sheep and goats. As well as infecting agricultural species, FMDV can be maintained within populations of certain wildlife species, such as African buffalo and wild boar, which act as a reservoir of virus capable of transmitting the virus to livestock (Alexandersen, Zhang et al. 2003, Alexandrov, Stefanov et al. 2013).

There are seven different FMD serotypes (O, A, Asia-1, SAT-1, SAT-2, SAT-3 and C) which tend to exist in regional virus pools, within which virus strains tend to circulate (Figure 1).



**Figure 1: The conjectured status and distribution of FMD, showing regional virus pools (Hamond 2012). Serotype C has not been detected since 2004.**

Following infection, FMDV replicates in the animal for between 2 and 21 days before the onset of clinical signs, during which time large amounts of virus may be shed into the environment. Typical signs of infection include vesicular lesions on the feet, mouth, tongue and udder, accompanied by fever and weight loss. In young animals, the virus can cause fatal myocarditis. Even when an animal recovers from infection, long-term morbidity is common, and up to 50% of recovered ruminants continue to harbour live virus in the absence of clinical signs. There is therefore the potential for these

carrier animals to transmit FMDV to other livestock, and for this reason their existence has far-reaching consequences for both the epidemiology of FMD and for trade with epidemic-recovered or endemic countries.

Thus the impacts of FMDV can be understood on two levels:

#### Direct

1. High mortality in young stock.
2. Reduced productivity including milk yield, fertility, growth rate, and traction power (where beasts of burden are used).
3. Change in herd structure with a need to maintain more breeding adults for the same level of output (for example, milk, young-stock, meat).

#### Indirect

1. Cost and impact of disease control including movement restrictions, market closures, vaccination costs and culling.
2. Control measures may impact on other industries including tourism.
3. Loss of access to lucrative livestock and livestock product export markets.
4. In endemic regions, FMD prevents the use of high productivity breeds that are more susceptible to the disease.

Countries or zones are divided into three categories depending on their FMD status: FMD-free without vaccination, FMD-free with vaccination and endemic. Each category will feel the threat and impact of FMD differently, and so have different priorities in terms of research, prevention and disease control.

For FMD-free countries/zones the emphasis of management is on reducing the risk and impact of virus incursions. Risk reduction is intimately linked to FMD incidence in both neighbouring and trade-partner countries, and is determined largely by a region's ability to manage the flow of animals and their products from these countries. Should an incursion occur, early detection is crucial, followed by rapid implementation of an effective control strategy. Once the outbreak has been controlled the focus moves to surveillance, which is required to identify remaining sources of infection, to prove freedom from disease, and eventually to regain international trading rights. Vaccination may be used

to assist outbreak control and therefore vaccine banks must be constantly maintained and frequently updated to maximise efficacy against currently-circulating strains.

Countries that are FMD-free with vaccination have similar interests, but may have different priorities in terms of vaccination, with long-term immunity being of particular relevance. A dependence on regular vaccination brings several problems of its own: differentiating infected from vaccinated animals (DIVA) by means of serology remains a challenge, and vaccinated animals may become sub-clinically infected thereby potentially masking an outbreak situation. In addition, the financial burden of vaccine provision, distribution and repeated gathering of livestock for inoculation is considerable.

In endemic countries there is an urgent need for potent, quality-assured vaccines that induce longer immunity and can be produced in vast quantities, at low cost. As the majority of FMD research is conducted in more affluent countries, which are also often FMD-free, our knowledge of controlling epidemics is considerably greater than that of managing endemic FMD. Accordingly, in these regions, control strategies are often sub-optimally designed and poorly implemented. This is a pressing concern as FMD production losses have the greatest impact on the world's poorest countries, where more people are directly dependent on livestock (Figure 2).

The annual global impact of FMD in terms of visible production losses and vaccination costs in endemic regions alone amounts to between 6.5 and 21 billion USD, while outbreaks in FMD-free countries and zones cause additional losses of >US\$1.5 billion a year (Knight-Jones and Rushton 2013). FMD is frequently listed as the most economically important disease of livestock in many developed and developing countries (Perry and Randolph 2003, Perry and Rich 2007, Perry and Rich 2007, Perry, Sones et al. 2007, Perry and Grace 2009, Rushton and Knight-Jones 2012).

Vaccination is now a component of every control program for FMD, whether in response to endemic disease or an incursion into a previously FMD-free region. However, most countries remain reliant on FMD vaccines that have changed relatively little over the last 30-40 years; they provide limited cross-protection against different viral strains, fail to induce sterile immunity and require regular boosting to maintain protective efficacy. The vaccines themselves are expensive and risky to produce, requiring the culture of large amounts of live virus for inactivation, and, once formulated, need cold-chain storage to point-of-use. Extensive research into the design of novel vaccines and improvements to current approaches is beginning to address these issues (see section on Vaccines within Controlling the Disease).

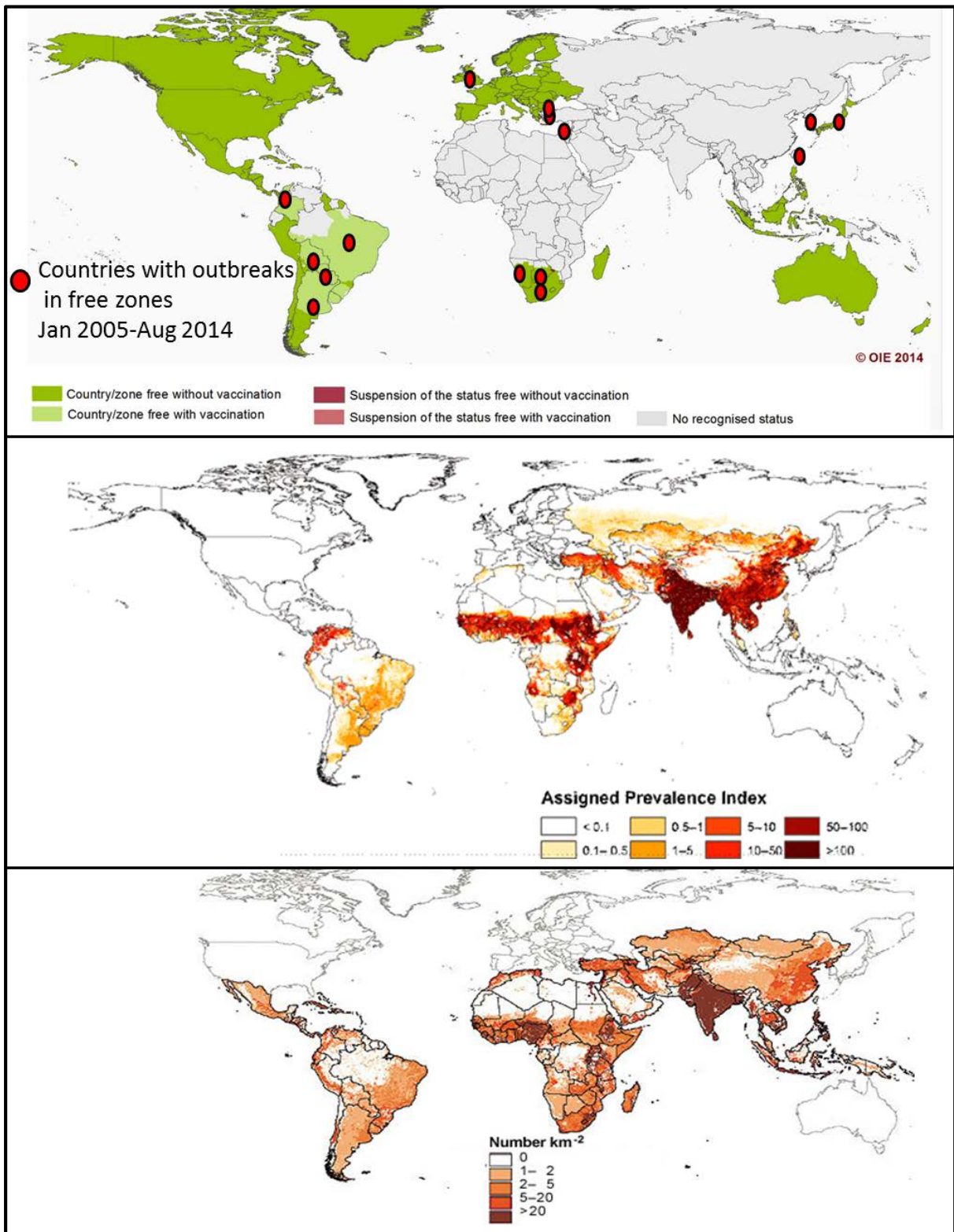


Figure 2: Upper panel – August 2014, OIE global FMD status, with recent outbreaks in free zones identified. Middle panel - global burden of FMD in cattle in 2008 (burden in sheep and goats has a similar distribution). Prevalence index based on estimates of incidence, population distribution and other risk factors, adapted from (Sumption, Rweyemamu et al. 2008). Note progress in South America since 2008 [compare with upper panel]. Lower panel - density of poor rural livestock keepers from (Thornton, Kruska et al. 2002). Central America, parts of South East Asia and some areas in South America are the few exceptions where FMD was not present in poor livestock keeper populations.



The long-standing challenges to effective control of FMD reflect gaps in our knowledge of the virus and the disease. The events immediately following exposure of the host to the virus remain poorly characterised, as do aspects of host species' immune systems and responses to the virus. How the virus' tropism for different host species, individual animals and tissues within those animals is determined, is as yet unclear. Accordingly, many of the gaps highlighted in this report are within the areas of basic research into FMDV-host interactions at the molecular and cellular level. Coupled with detailed immunological studies in host species, these studies will enable rational design of improved vaccines formulated in a way that will facilitate their widespread use. As ongoing research projects are completed and followed by appropriately conducted studies in the areas highlighted here, we believe that there is every reason for optimism that our ability to control FMD will be substantially improved in the near future. This will, however, depend entirely on significant funding to maintain and advance the most promising research and development programs.

In the following section we present a review of recent literature on FMD research and updates of ongoing work at GFRA and EuFMD institutes. To aid understanding, this information is divided into research topic areas, which themselves fall under the broader headings of either Controlling the Disease or Understanding the Virus.

## Controlling the Disease

### Global FMD update: 2011-14

The global FMD landscape has undergone several shifts since publication of the 2011 GFRA report. FMD SAT-2 outbreaks in North Africa and the Middle East caused significant losses and an international crisis in 2012, which was exacerbated by limited efficacy of the SAT-2 vaccine used. In 2014, serotype O outbreaks in non-endemic regions of North Africa have been a cause for concern: in Southern Africa, outbreaks continue to occur within long-established, and previously successful, FMD control programmes that are based on zonation, vaccination and exclusion of FMD endemic wildlife. This has been disastrous for the beef export industry.

The most recent FMD outbreak in the EU occurred in 2010/11, and was associated with wild boar infected with an Asia-1 strain from Turkey. This outbreak highlighted the previously-underestimated role of wildlife in this region and revealed shortfalls in vaccine matching and potency of some vaccine banks in FMD-free countries. Sporadic outbreaks continue to be reported in Russia.

In Asia, large outbreaks have occurred in the previously FMD-free countries of Japan (2010/11) and South Korea (2010/11 and 2014) as a result of virus spill-over from epidemics within neighbouring endemic countries. Encouragingly, the Philippines have now achieved FMD-free status, which should have considerable benefits for the region. India and China have both prioritised FMD control and are vaccinating on a large scale; this is a considerable challenge given the scale of quality-controlled vaccine production required and the necessity for high coverage across vast areas. Greater still is the challenge of implementing acceptable, yet effective, movement controls and biosecurity measures in smallholder systems.

There is cause for optimism in South America, where no FMD outbreaks have been reported since early 2012, with more and more countries/zones obtaining official FMD-free status. The know-how amassed within these control programmes could be utilised by those elsewhere attempting to control the disease through mass vaccination, but effective means of disseminating this valuable knowledge remain to be developed.

## Epidemiology and control

### From the 2010 Gap Analysis:

The priority aim was to produce analytical tools to support decision-making, with an emphasis on developing:

1. Anomaly detection methods to identify outlier events.
2. Prediction models for identification of genetic variants of viruses, to predict severity, duration, and likelihood of transmission of disease, and to evaluate the degree of success of control and prevention interventions.
3. Epidemiological models that project spread of disease in a defined region under various control strategies and that can be used in developing disease control programs and for active surveillance sampling.

### Literature review

#### *Improved models*

Since the 2011 report, 111 studies of FMD epidemiology have been published, almost half of which described advances in mathematical modelling. Most used transmission models to predict the consequences of outbreaks in FMD-free countries, estimate resource requirements and compare different control options, particularly the impact of vaccination [see section on control of epidemics in disease free countries] (Backer, Engel et al. 2012, Backer, Hagenaars et al. 2012, Hagerman, Ward et al. 2013, Garner, Bombardieri et al. 2014). One study combined data on FMDV ecology and maintenance with a modelling approach to estimate the relative roles of domesticated animal species compared to deer and wild boar, with reference to a Bulgarian outbreak in 2011 (Dhollander, Belsham et al. 2014), revealing the potential for wildlife hosts in similar climatic zones to contribute substantially to the spread of disease.

Several papers used risk analysis to assess the chance of a FMDV incursion when importing livestock and commodities. Simulation of theoretical outbreaks in France revealed the potential positive impact of improved surveillance protocols, and in particular, the importance of having specialist veterinarians available to support diagnosis as soon as FMD was suspected (Rautureau, Dufour et al. 2012).

Models developed for epidemics are largely not appropriate for use in endemic situations, but developing models to deal with the complexity of endemic FMD is enormously challenging. A recent

study identified important factors influencing control in a vaccinating endemic region, suggesting that duration of natural immunity, rate of vaccine induced antibody waning, and the rate of disease re-introduction are key to the success of vaccine strategies. Moreover, the efficacy of prophylactic, as opposed to ring, vaccination was highlighted in this model, as was the importance of the duration of natural immunity in determining size and frequency of disease outbreaks (Ringa and Bauch 2014).

A few publications compared and reviewed the suitability of different modelling strategies (Sanson, Harvey et al. 2011, Tildesley and Ryan 2012, Flood, Porphyre et al. 2013, Halasa, Boklund et al. 2014); a retrospective evaluation of model performance illustrated the impact of poor control decisions based on models designed using inaccurate data (Mansley, Donaldson et al. 2011), highlighting the importance of precise knowledge of pathogenesis and transmission in different species under varied conditions.

#### *New data to inform better modelling*

→ Consideration of the combined outcomes of various studies by meta-analysis, as illustrated by (Bravo de Rueda, Dekker et al. 2014) supports the need for improved parameterisation and the development of more robust epidemiological models.

Model accuracy is limited by input data quality, which in turn comes from both laboratory studies and from field epidemiology. Field studies constituted about a quarter of FMD epidemiology publications, largely conducted in countries where FMD is endemic. Several were descriptive or risk factor studies based on FMD sero-surveys of countries in the early stages of FMD control (Dukpa, Robertson et al. 2011, Abubakar, Arshed et al. 2012, Ayelet, Gelaye et al. 2012, Dukpa, Robertson et al. 2012, Kibore, Gitao et al. 2013), sometimes looking at wildlife (Bolortsetseg, Enkhtuvshin et al. 2012, Alexandrov, Stefanov et al. 2013, Jori, Caron et al. 2014, Mkama, Kasanga et al. 2014). Consistent, rigorous sampling coupled with standardised design and analysis would allow clearer estimation of the burden of disease and accurate comparison of that burden between different sero-surveys.

#### *Evaluation of efficacy of different control methods in endemic countries:*

Field studies are starting to be used to assess vaccine performance (Brito, Perez et al. 2011, Knight-Jones, Bulut et al. 2014). A recent meta-analysis of 28 studies estimating the efficacy of vaccination in China suggested that more than 70% of animals from government-targeted species were adequately protected against serotypes Asia-1 and O (Cai, Li et al. 2014).

→ Guidance on target vaccine coverage and efficacy should be updated using modern approaches.

→ The ability to monitor population immunity would be enhanced by the identification of stronger correlates of protection, above/more reliable than the virus neutralisation test (VNT). Current knowledge of correlates is largely limited to the first month after vaccination with homologous strains. Within a vaccination programme, long term protection against heterologous strains is important.

→ Improved tests to detect FMDV carriers are required, isolation of live virus from oro-pharyngeal region is time consuming and unreliable.

### *Surveillance*

Several studies also assessed surveillance data (Madin 2011, Kasanga, Sallu et al. 2012) and its use in disease management (Willeberg 2012). In a field based surveillance study, a combination of non-structural protein (NSP) antibody surveillance of pigs at livestock markets followed by clinical investigation was used to identify FMD infected farms in Taiwan (Chen, Lee et al. 2011). An important, yet hard-to-quantify issue is behavioural factors affecting disease reporting and control. Attempts have been made to understand the barriers to compliance for farmers in Texas (Delgado, Norby et al. 2012), and Bolivia (Limon, Lewis et al. 2014). Taken together, these studies effectively highlighted the differences between farming populations in the two countries, and the importance of understanding local cultural and social factors that influence the agricultural community's motivations to report FMD outbreaks.

### *Molecular studies*

Substantial improvements in our ability to accurately model FMD have come via molecular epidemiological studies that have been able to link outbreaks caused by closely related viruses (Ahmed, Salem et al. 2012, Hui and Leung 2012, Hall, Knowles et al. 2013, Wright, Knowles et al. 2013), as well as estimate the likely roles of various transmission routes in both the UK 2001 (Morelli, Thebaud et al. 2012) and Bulgarian 2011 outbreaks (Valdazo-Gonzalez, Polihronova et al. 2012).

### *Challenge studies*

Challenge studies have refined knowledge of key epidemiological parameters including: the point of onset of clinical signs during infection in cattle (Chase-Topping, Handel et al. 2013); the relationship between onset of clinical signs and the FMD transmission window in cattle (Charleston, Bankowski et al. 2011); the ability of sheep to transmit the virus to cattle ["the results indicated that in a mixed population of sheep and cattle, sheep play a more limited role in the transmission...than cattle." (Bravo de Rueda, de Jong et al. 2014)]; the potential of wild boar (Breithaupt, Depner et al. 2012), or buffalo

(Madhanmohan, Yuvaraj et al. 2014) to act as hosts; and the optimal use of full-genome sequencing to understand transmission pathways during epidemics (Juleff, Valdazo-Gonzalez et al. 2013, Orton, Wright et al. 2013). Field data have also been combined with transmission experiments (Hagenaars, Dekker et al. 2011, Sanson, Gloster et al. 2011) and modelling approaches (Chis Ster, Dodd et al. 2012) to generate improved data for incorporation into next-generation models.

### *Descriptive studies*

Other field epidemiology papers described eradication campaigns and factors contributing to success (Windsor, Freeman et al. 2011, Naranjo and Cosivi 2013). Such reports are useful to endemic countries attempting improved FMD control, where informed control policy guidance is sometimes limited. Quantitative evaluations of the progress and effectiveness of ongoing control programmes in endemic countries were few and far between and would be similarly valuable in informing future decision-making.

Similarly important are the descriptions of recent outbreaks (Muroga, Hayama et al. 2012, Cho and Chu 2013), including lessons learned and reviews of regional or national FMD situation, including disease epidemiology (Swallow 2012) and control strategies (Parent, Miller et al. 2011, Leon 2012, Ding, Chen et al. 2013, Ferguson, Cleaveland et al. 2013, McReynolds and Sanderson 2014).

→ Facilitating the sharing of knowledge between those with recent experience of FMD eradication in endemic countries and individuals responsible for similar attempts in their own nation would minimise the repetition of mistakes and likely improve overall outcomes.

→ A problem for most endemic countries is that they are unable to improve biosecurity and movement restrictions, given the small holder systems that predominate with dependency on common grazing and frequent trading of livestock to provide income for many people. More potent vaccines may control FMD despite poor biosecurity, however, this has yet to be assessed.

### *Breed resistance*

Antibody response to commercial tetravalent vaccine was significantly impacted by breed (Di Giacomo, Brito et al. 2013). Breeding for both productivity and resistance to FMD may represent a complimentary approach to conventional control measures in endemic regions. One study provides evidence that MHC type can determine clinical protection from disease following inoculation of virulent FMDV in naive Wanbei cattle (Lei, Liang et al. 2012).

## Control of epidemics in disease-free countries

In terms of FMD control policy, much of the efforts of FMD-free countries is concerned with minimising the risk of a virus incursion and minimising the impact of an outbreak should one occur. This comprises several different aspects, including identifying high risk activities likely to increase the chance or severity of an outbreak, predicting how an outbreak is likely to behave and deciding on the most effective control policy for a range of situations. Contingency planning should ensure that resources, skills and legislative capacity are sufficient. Ground testing, including simulation exercises, should be performed to assess control measures for efficiency, feasibility, compliance and disruption to the farming sector and other areas of the economy.

### *Modelling*

Predictions about outbreaks and control measures, used to inform contingency plans, have become heavily reliant on modelling. This is due to the complex nature of disease transmission within the livestock population [also mentioned in the epidemiology section].

For example, prediction models have suggested that the final scale of an outbreak is strongly correlated with the situation after two weeks, and this could inform decisions on whether or not to scale up control efforts at this early stage of an outbreak (Halasa, Willeberg et al. 2013). Elsewhere fault tree analysis has been used to identify potential weaknesses on control strategy (Isoda, Kadohira et al. 2013).

### *Vaccination*

The role of vaccination has been modelled extensively. In the Netherlands, it was estimated that in areas of high farm density, ring vaccination would halt an epidemic as rapidly as pre-emptive culling, whilst dramatically reducing the number of farms culled. However, if large numbers of pig farms require vaccination, vaccine supplies will be insufficient as pig farms contain many more individuals than cattle farms. Not vaccinating pigs did not appear to impact significantly on epidemic size (Backer, Hagenaars et al. 2012). However, findings from models are likely to be specific to the setting evaluated. The same model suggested that in areas of low farm density, culling detected farms only, without vaccination, would be sufficient, assuming infected farms are detected 6-10 days for cattle and pigs, with lower detection rates in sheep and goats, and culling taking place a day after detection (Backer, Hagenaars et al. 2012). These results were also used to assess the most efficient post-outbreak surveillance strategy (Backer, Engel et al. 2012). In Switzerland, vaccinating a wide area around affected herds was considered to only be beneficial during a widespread, non-localised

epidemic, while failure to restrict movements and delayed culling were deemed critical, highlighting the importance of preparedness and the capacity of veterinary services (Durr, Fasel-Clemenz et al. 2014). A simulation of FMD in Germany found widespread ring vaccination preferable to pre-emptive culling (Traulsen, Rave et al. 2011). Furthermore, as large numbers may be culled for welfare reasons as a result of movement restrictions within vaccination zones, this should be considered during outbreak resource requirement planning (Hadorn, Durr et al. 2013).

One paper sought to clarify FMD vaccination policy in the USA by presenting several key decision makers with an outbreak scenario to establish when and why they would implement emergency FMD vaccination (Parent, Miller et al. 2011). Elsewhere, the influence of different stakeholders on control policy found that in France a vaccination based strategy was always the preferred national policy despite regional differences (Marsot, Rautureau et al. 2014).

### *Livestock movements*

The enormous scale of livestock movements is responsible for much of the impact of notifiable disease outbreaks. In an attempt to mitigate this, more restrictive routine movement regulations were implemented in many countries in the wake of the devastating 2001 outbreak in Northern Europe. However, a Dutch study found that in the long term the scale of trading and movements returned to pre-outbreak levels despite increased regulation (Brouwer, Bartels et al. 2012). However, following past outbreaks in the UK, routine movement standstill regulations have been maintained as part of the FMD control policy as a key component of limiting spread of the virus.

→ Thorough policy evaluation following outbreaks is rarely performed in most countries and should be encouraged to inform improved decision-making.

Movement data are crucial inputs for most livestock models. Where they are lacking, the utility of models may be limited. In turn, the impact of movement restrictions is a common model output. The effectiveness, timeliness and compliance of movement restrictions is typically found to be of paramount importance (Buhnerkempe, Tildesley et al. 2014). In addition, effective tracing of livestock movements has also been shown to drastically reduce the impact of outbreaks in free countries (Hagerman, Ward et al. 2013, Mardones, Zu Donha et al. 2013).

The need for farmers to both report disease and to undertake legislated control measures is critical for outbreak control. In Texas, USA, farmer compliance was likely to be suboptimal in terms of case reporting, collecting cattle for examination and observing a movement standstill during an outbreak



(Delgado, Norby et al. 2012). Understanding factors underlying non-compliance may aid design of more widely-accepted methods, or of appropriate incentives to support the FMDV control effort.

### *Preparedness*

Disease outbreaks are often initially detected at abattoirs, highlighting the importance of frequent inspections by well-trained inspectors (Hernandez-Jover, Cogger et al. 2011). Unfamiliarity with exotic diseases at all levels, including farmers, can also contribute to a lack of preparedness. To help address this, EuFMD have run real-time training courses in endemic countries where government veterinarians, mostly from free countries, obtain first-hand experience of FMD outbreaks (Gardiner 2013). These courses equip participants with diagnostic sampling and field epidemiology skills necessary for vets in the field during FMD outbreaks. Importantly they expose vets who have often never seen a case of FMD to real outbreaks, providing training in clinical diagnosis and biosecurity <sup>1</sup>.

→ Awareness and preparedness training should also involve livestock keepers and other relevant stakeholders within livestock value chains.

The potential role of FMD in bioterrorism has been considered by some governments due to the relative ease with which highly contagious material can be obtained from outbreaks in endemic countries and the severe economic consequences of outbreaks in disease-free countries (Farsang, Frentzel et al. 2013). Some governments have used prediction models to estimate resource requirements during an outbreak and how availability of manpower influences the impact of an outbreak (Garner, Bombardieri et al. 2014). One study suggested that for outbreaks in Australia, stamping out would be effective, however, if human resources were limited then a vaccination strategy may be preferable to culling alone (Roche, Garner et al. 2014). Others have used expert opinion and group consultations to explore some of the logistics of outbreak containment. One study concluded that depopulation of a large feedlot would be difficult to complete in a humane and timely fashion (McReynolds and Sanderson 2014).

Many countries have described their contingency plans, sometimes specifying where weaknesses exist (Diez and Styles 2013, Ding, Chen et al. 2013). These plans are discussed in detail with stakeholders, including government officials, livestock leaders, scientists and figures in the wider industry. Of course control of transboundary diseases requires not only within-country cooperation and coordination, but

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<sup>1</sup> <http://www.fao.org/ag/againfo/commissions/eufmd/commissions/eufmd-home/eufmd-in-action/training/en/>

wider international and regional collaboration (Corrales Irrazabal 2012, Sumption, Domenech et al. 2012).

### *Environmental impact*

The environmental and public health impact of the mass usage of disinfectant caused concern in the Republic of Korea (South Korea) (Kim, Shim et al. 2013) as did large scale burial of slaughtered pigs (Yang, Hong et al. 2012). Welfare concerns during mass culling were also an issue in this outbreak.

### **Research updates**

Various institutes have active FMD epidemiology research programmes. The Pirbright Institute has continued its work on the field evaluation of FMD vaccination programmes, both against field challenge and through post-vaccination immunity. IZSLER, in Italy has also helped endemic countries with sero-surveys to measure population immunity and post-vaccination response, and to assess disease burden and risk factors. The National Institute of Agricultural Technology (INTA) has examined population immunity in Argentina.

CIRAD (Centre de Coopération Internationale en Recherche Agronomique pour le Développement), CODA (Centrum voor Onderzoek in Diergeneeskunde en Agrochemie), Wageningen, Onderstepoort Veterinary Institute (OVI) and University of Pretoria, and the University of Glasgow are several of the member institutes trying to further understand the role of wildlife in FMD epidemiology, further discussed in the wildlife section. Other work by CODA has confirmed the importance of NSP sero-surveillance for the identification of FMDV transmission in the field. CIRAD has also evaluated the efficiency of FMD surveillance in South East Asia, assessing participatory approaches to surveillance and control. As well as descriptive studies of FMD epidemiology in Tanzania, working with the Tanzanian Veterinary Laboratories Agency, the University of Glasgow has collaborated closely with The Pirbright Institute on various quantitative analyses of molecular data and how this can provide insights into virus ecology and epidemiology, including how viruses evolve within and between hosts and how genetic variation relates to population burden of infection.

CSIRO-Australian Animal Health Laboratory (AAHL) has helped to develop vaccine control strategies in the event of an outbreak in Australia. This work has also attempted to identify the strains that pose the biggest threat to the Australian livestock economy.

Mirroring the increasing acceptance of models in policy planning, the Technical University of Denmark (DTU) has modelled hypothetical FMD outbreaks in Denmark. The University of Minnesota is looking at FMD control in swine if an outbreak occurred in the US.

The World Conservation Society-Animal & Human Health for the Environment and Development (WCS–AHEAD) has helped look at value chain approaches to manage FMD and food safety risks in the beef production chain in Namibia. Elsewhere they have used modelling approaches to understand the epidemiology of SAT (Southern African Territories) serotypes in African buffalo in southern Africa.

With assistance from the USA, Pakistan research institutes have investigated FMD distribution and the role of persistently infected cattle and water buffalo. Descriptive studies are also underway in Cameroon and Uganda. In Nigeria descriptive studies have been used as a means of increasing diagnostic capacity.

In the Netherlands, Wageningen University have been particularly active. One study found that environmental virus accounts for 1/3 of transmission in housed cattle but the route of infection into the host remained unknown. They also found that small ruminants were as susceptible as cattle but less infectious. Modelling studies estimated that a 2-5km ring vaccination policy could contain an outbreak and confirmed that minimising the duration of an outbreak was a critical aspect of limiting economic impact. As part of the EU FMD-DISCONVAC project, managed by CODA, they have been involved in the validation of FMD control models, something of huge importance given the increasing acceptance and reliance on models to advise policy.

Plum Island Animal Disease Center (PIADC) has been involved in multiple projects in the US as well as Africa and Asia. Some projects are technical, such as the FMD bioportal to assist international sharing of information and modelling studies; other projects are field-based, including descriptive epidemiological studies and evaluation of the role of carriers in the field.

For a summary of research updates from contributing institutions, see appended Table 2.

### **Research priorities**

Although the field of FMD modelling is progressing, validated best practices have not yet been established. The extent to which generic spread models can be used in different settings also needs to be determined. Greater ground testing of real time decision support tools, including those capable of estimating cost-effectiveness of different strategies is also required.

Developments are also required in surveillance methods, particularly when confirming disease free status in vaccinated populations.

Clear guidance, knowledge sharing and evidence based policy development are required to assist endemic countries to control FMD, particularly those with large smallholder farming sectors.

As molecular and genetic technologies become cheaper and more powerful, so does their use in epidemiology. This progress needs to continue as major breakthroughs could be imminent. The incorporation of these tools into practical disease control should be encouraged.

Basic field epidemiology is under-utilised in the evaluation of control programmes, particularly in endemic countries.

Approaches for identifying high risk strains for incursion and impact in FMD free countries should be developed.

FMD has not been controlled by vaccination alone in the absence of effective biosecurity measures and efficient rapid diagnosis. Workable control strategies suitable for developing countries in the early stages of FMD control need to be developed and evaluated.

## **Wildlife and FMD control**

### **Literature review**

#### *Roles of wildlife species*

A major obstacle to control in certain FMD-endemic regions is the role played by wildlife species in maintaining and spreading the disease. Publications between June 2011 and June 2014 have largely focused on three different aspects of FMD in wildlife: characterisation of FMD strains, burden of infection or susceptibility within specific species and populations; understanding transmission dynamics within wildlife populations, and between wild and domestic animals; and defining optimal control strategies for those regions where wildlife populations play a significant role in the epidemiology of FMD.

In 2010/2011 FMD-infected wild boar (*Sus scrofa*) were detected in FMD-free Bulgaria. Infection was also detected in domestic livestock. Further investigation revealed clustering of small groups of

seropositive animals in both wild boar and deer populations, suggesting that these species have a limited capacity to maintain and spread FMDV (Alexandrov, Stefanov et al. 2013). Surveys of wild boar in Turkey subsequently determined a sero-prevalence of 20% in animals within endemic regions (Khomeenko, Alexandrov et al. 2012), although finding a clinically-infected animal was extremely rare. A modelling study that considered this information, along with the results of experimental studies of FMD infection in wild boar (Breithaupt, Depner et al. 2012), supported the hypothesis that the wild boar and deer populations on the Bulgarian-Turkish border were unable to maintain FMD infection in the absence of infection in domestic animals, but highlighted the possibility that they could play an important role in transmission during future outbreaks under different conditions (Dhollander, Belsham et al. 2014).

→ There is a need to monitor populations of susceptible wildlife species, as well as factors affecting/likely to predict changes in their numbers/geography, e.g. wild boar populations have grown enormously in parts of Europe in recent decades, and we must be responsive to the increased risk they pose during potential outbreaks.

The ability of African buffalo (*Syncerus caffer*) to harbour a number of pathogens that are transmissible to domestic livestock creates a costly and complex problem for both farming and conservation communities (Michel and Bengis 2012, Caron, Miguel et al. 2013). Although there is some debate, it seems probable that African buffalo are the only wildlife species that play a significant role in the maintenance of FMD within the region (Weaver, Domenech et al. 2013). Probang samples from African buffalo in Tanzania, Zambia and Mozambique contained FMDV serotypes SAT 1-3, suggestive of their maintenance within the buffalo populations (Weaver, Domenech et al. 2013, Kasanga, Dwarka et al. 2014). A prospective serological study of cattle grazing adjacent to wildlife in Zimbabwe also detected sero-conversion to FMD serotypes carried by buffalo, but in the absence of clinical signs, indicating the need for close monitoring as part of effective FMD surveillance in these settings (Jori, Caron et al. 2014). Another study in South Africa found that cattle made far more contacts with buffalo than with other wildlife species (Brahmbhatt, Fosgate et al. 2012), providing further evidence linking the two species in maintaining FMDV infections in the wider ungulate population.

A review of FMD susceptibility in camelids concluded that whereas Bactrian camels are susceptible to FMD, dromedaries are not; furthermore, new world camelids of South America have limited susceptibility and a low risk of transmitting the infection (Wernery and Kinne 2012). A serological study of Mongolian gazelles (*Procapra gutturosa*) and livestock on the eastern Steppe of Mongolia also concluded that FMD spills over to gazelle populations from outbreaks in domestic livestock, and control efforts need to focus on the latter (Bolortsetseg, Enkhtuvshin et al. 2012).

The ability of feral pigs (*Sus scrofa domesticus*) and white-tailed deer (*Odocoileus virginianus*) to be infected and transmit FMD was confirmed in experimental studies (Mohamed, Swafford et al. 2011, Moniwa, Embury-Hyatt et al. 2012). The role that deer and feral pigs could play in epidemics in the USA was further explored in a modelling study, but our knowledge of FMD transmission in wildlife populations is limited, and consequently their role in FMD outbreaks is hard to predict (Ward, Laffan et al. 2011).

#### *Wildlife considerations and disease control*

Traditional strategies for controlling FMD at the interface between wildlife and livestock have had a negative impact on wildlife, as well as on some human populations. This is particularly true when fencing has been used to segregate wildlife and livestock from FMD control zones. There have been calls for more balanced approaches that safeguard wildlife populations and do not disrupt the ecosystems upon which many people depend for a livelihood: in order to achieve this, a better understanding of FMDV circulation between wild and domestic species is required, as is an appreciation of the economic value of wildlife (Ferguson, Cleaveland et al. 2013). See commodity based trade in socio-economics and international standards section.

### **Research updates**

Much is being done to increase our understanding of the role of wildlife in FMD epidemiology. In Europe, interest has centred on wild boar (*Sus Scrofa*) after outbreaks in Thrace and widespread seropositivity detected in Turkey. A study by the European Food Safety Authority (EFSA) concluded that FMD would not be maintained by wild boar in Thrace (EFSA Panel on Animal Health and Welfare (AHAW) 2012). A modelling study highlighted the limitations of passive surveillance and reliance on hunting for surveillance in wildlife when compared to active sample collection (European Food Safety Authority 2012).

In sub-Saharan Africa there have been efforts to further understand and quantify the risk of FMDV transmission from African buffalo to domestic species. More importantly, efforts are required to define conditions under which FMDV-free beef can be produced in regions where the virus is endemic in wildlife, without impacting on wildlife and wildlife tourism.

For a summary of research updates from contributing institutions, see appended Table 2.

## Research priorities

A better understanding of the role of wildlife in FMD maintenance and transmission is required in various endemic settings.

This would provide evidence to inform policy on the role of wildlife in official FMD status.

## Socio-economics and international standards

### Literature review

The benefits of controlling FMD in developed livestock sectors that are able to export livestock and their products is so apparent that detailed economic studies have not traditionally been required. The benefits of FMD control elsewhere are more controversial and less well understood. In the endemic setting and in less developed countries, the case for FMD control must be made by detailed and robust economic studies in order to justify funding. This is recognised by the PCP-FMD, which states that such studies should be performed during the early stages of a control programme (EuFMD/FAO/OIE 2012). Despite this, the field receives little attention and methods for estimating the many complex aspects of FMD impact are not established.

The fourteen identified peer-reviewed publications since June 2011 include a handful of studies using field studies to estimate the economic impact of FMD on smallholders in endemic countries, mostly in South-East Asia (Shankar, Morzaria et al. 2012, Ferrari, Tasciotti et al. 2013, Nampanya, Khounsy et al. 2013, Young, Suon et al. 2013, Jemberu, Mourits et al. 2014). FMD control in endemic countries has traditionally been deemed a lesser priority, particularly for countries without the potential to benefit from FMD free export markets. These studies found that impact was significant but variable from region to region. One study in South-East Asia estimated that smallholder outbreaks resulted in a reduction in household income of 4-12% (Shankar, Morzaria et al. 2012), in parts of Laos losses in FMD affected households may account for 60% of annual household income (Nampanya, Khounsy et al. 2013). In many societies cattle are valued as assets even when not traded for financial gain, but rather as a resource that can be “cashed in” at times of need. Thus FMD can have big impact on livelihoods even when livestock are not raised in a commercially intensive way.

One paper looked at the effect of FMD on market prices in the Philippines and found that: pig farm and pork wholesale prices dropped 11.8% and 15.7%, respectively; somewhat surprisingly, chicken

farm and wholesale prices declined by 21.1% and 14.2%; and that the margins of pig and chicken traders were also adversely affected (Abao, Kono et al. 2014).

Some modelling studies incorporated economic consequences when assessing national effects of FMD (Boklund, Halasa et al. 2013, Martinez-Lopez, Ivorra et al. 2014). Although this can be uncertain, economics is critical in policy making and must not be neglected by researchers.

There was one review of the global impact of FMD which estimated that in endemic countries FMD causes a global economic impact, due to production losses and vaccination costs alone, of around US\$6.5 to 21 billion per year (Knight-Jones and Rushton 2013). Two papers looked at theoretical and technical issues associated with animal health economics (Hasler, Howe et al. 2011, Gosling, Hart et al. 2012). In addition there were several studies on disease prioritisation (Chatikobo, Choga et al. 2013, Jibat, Admassu et al. 2013, Onono, Wieland et al. 2013), often finding FMD to be deemed a priority by farmers and policy makers in both the developed and developing world, although regional variation exists. A handful of additional reports on FMD impact in endemic countries were published in the grey literature (Ashenafi 2012, Botswana parliamentary inquiry 2013, Limón, Guitian et al. 2014).

#### *International standards for trade*

The standards required to trade with official FMD free status are prescribed by the OIE. The waiting period required before livestock exports can resume after an outbreak in a free country has been eradicated vary depending on whether vaccination was used and the fate of the vaccinated animals, i.e. whether they have been removed or not. Some have argued that given, sufficient surveillance, a 3 month waiting period should apply after vaccination regardless of whether vaccinated animals are later culled or not (Barnett, Geale et al. 2013, Geale, Barnett et al. 2013). Of particular importance is the ability to prove FMD freedom within a vaccinated population, usually by clinical and passive surveillance supplemented with extensive sero-surveillance using imperfect diagnostic tests. Possible approaches and problems have been considered (Caporale, Giovannini et al. 2012), however, challenges remain. Furthermore, unlike for diagnostic tests, clinical detection of FMD has not been evaluated in terms of sensitivity and specificity and this limits the certainty we can have in disease free certification (Knight-Jones, Njeumi et al. 2014).

Commodity-based trade is based on the principle that the FMD-free status of a product can be assured without the need for complete FMD freedom within a large geographic zone including wildlife (Thomson, Penrith et al. 2013, Thomson, Penrith et al. 2013). The benefit of this approach is that not only does it remove the need to enclose and restrict the movements of people, livestock and wildlife, it also reduces the impact of FMD outbreaks on distant farmers that would otherwise lose access to



export markets though loss of zonal FMD free status, making livelihoods more dependable and trade less volatile. Furthermore, by increasing the accessibility of lucrative export markets, more producers have a stronger incentive to control FMD.

### Research updates

Collaborations between the University of Sydney and local partners in South-East Asia have looked at FMD impact in Smallholder systems, an area of interest to international donors. This area is being reviewed by researchers at The Royal Veterinary College and the International Livestock Research Institute (Knight-Jones and Rushton 2014). Although mortality from FMD is low, the continued, and widespread, high disease incidence clearly leads to a heavy burden of disease at the population level. However, FMD impact in smallholder systems has received relatively little attention and more studies are needed.

Studies at the Friedrich-Loeffler-Institute (FLI) and The Pirbright Institute have also looked at bio-safety of certain livestock products to begin to understand the FMD risk associated with international trade.

For a summary of research updates from contributing institutions, see appended Table 2.

### Research priorities

Improved understanding of farmer participation in disease reporting and control, including how best to use compensation as a tool for disease control.

Improved understanding of the impact of FMD and FMD control, particularly in endemic countries with limited potential to access export markets.

Real-time use of economic models to guide disease control during outbreaks.

Options for FMD control and status in wildlife endemic settings, such as commodity based trade, require ground testing.

## Vaccines

### From the 2010 Gap Analysis

Priority research aims stated were to:

1. Develop vaccinal needle-free strategies to induce mucosal as well as systemic responses in susceptible species
2. Develop vaccine formulations effective in neonatal animals with or without maternal immunity
3. Investigate the safety and efficacy characteristics of novel attenuated FMD vaccine platforms
4. Understand and overcome the barrier of serotype- and subtype-specific vaccine protection
5. Design and engineer second-generation immune refocused FMDV antigens
6. Improve the onset and duration of immunity of current and next generation FMD vaccines
7. Develop next generation FMD vaccines that prevent FMDV persistence
8. Invest in the discovery of new adjuvants to improve the efficacy and safety of current inactivated FMD vaccines
9. Develop vaccine formulations and delivery targeting the mucosal immune responses

### Literature review

The vaccine field is the best represented in terms of number of new publications since 2011, with many investigators conducting small scale trials of a range of innovative vaccine approaches. For a review of new vaccine technologies see (Rodriguez and Gay 2011). The licensing of the first FMDV vaccine for emergency use in the US has revolutionised the landscape, and an increasing appreciation of the importance of mucosal and cellular immune responses to successful vaccine strategies is filtering through into rational vaccine design studies. Moreover, several exciting developments in the arena of needle-free delivery systems have been made, but are yet to be translated to large-scale field condition trials.

#### *Novel/new vaccines*

At the point of writing the 2010 Gap Analysis, a new generation of Human adenovirus 5 (Ad5) derived FMD vaccines was under development by PIADC in conjunction with industry partners GenVec. In October 2012, the first such vaccine was licensed for use in cattle in the US under outbreak conditions.

The licensed vaccine comprises an adjuvanted formulation of replication-deficient human adenovirus 5 (Ad5) engineered to encode the FMDV structural proteins VP0, VP1 and VP3, alongside the viral protease 3C required for their cleavage from the polyprotein. In culture, the structural proteins will spontaneously assemble into empty capsids that retain much of the immunogenicity of inactivated whole virus. Employing recombinant adenovirus in place of live FMDV offers significant advantages for the security and ease of vaccine production, which has enabled the vaccine to be manufactured on the US mainland. Encoding only the FMDV proteins required for empty capsid assembly should also permit differentiation of infected from vaccinated animals (DIVA) by conventional detection of anti-NSP antibodies in recovering animals, or with the FMDV 3D ELISA that is being developed in parallel with the vaccine. Efficacy studies are reported to show protection from as early as 7 days post-vaccination in greater than 95% of cattle following a single immunization.

While the newly-licensed vaccine undoubtedly represents a major leap forward in the field and is well suited to meet the needs of an epidemic in the US, its potential benefits in FMD endemic regions may be limited as a result of, for example, its storage requirements. However, the empty capsid, or virus-like particle (VLP), strategy could be exploited in a different way: in place of producing recombinant virus with the aim of generating the VLPs *in vivo* following immunization, empty capsids can be synthesized *in vitro* as a vaccine antigen. Until recently, the problem with this approach was the cellular toxicity of FMDV 3C protein, leading to low/variable yields of capsid; we now know that yields of serotype O and A capsid from mammalian cells can be markedly improved through limiting the expression of 3C relative to P1-2A by a variety of means (Gullberg, Muszynski et al. 2013, Polacek, Gullberg et al. 2013). Using a novel construct encoding FMDV A P1-2A, with 3C frame-shifted to reduce its activity, researchers from the Pirbright Institute extended these findings into insect cells, where a high level of FMDV serotype A empty capsid expression was achieved with minimal toxicity. Importantly, the capsids produced were recognised by sera from both infected and vaccinated cattle (Porta, Xu et al. 2013). The same group then showed that by incorporating a mutation designed to stabilize the capsids during exposure to heat or low pH, they could produce a commercially-viable vaccine candidate able to induce strong humoral responses in cattle and sustained protection from challenge (Porta, Kotecha et al. 2013).

→ This approach could prove revolutionary for the production of FMDV vaccines in a safe and cost-effective way. Their efficacy in susceptible species other than cattle remains to be evaluated, and it will be necessary to understand their stimulation of non-humoral aspects of the immune response in order to fully optimize their design and formulation.

Several other groups have also shown promising results using various VLP strategies: FMDV Asia 1 capsids produced in *E. coli* protected cattle and swine from challenge, and stimulated FMDV-specific T-cell proliferation and IFN- $\gamma$  secretion in cattle (Guo, Sun et al. 2013); using mammalian cells grown in suspension culture may also prove an easily scalable method of empty capsid production for FMDV vaccines (Mignaqui, Ruiz et al. 2013). Furthermore, targeted modifications to the FMDV capsid sequence can render vaccine viruses more stable as well as facilitating their adaptation to tissue culture, for example, introducing lysine at VP1-110 significantly enhanced cell culture adaptation of the virus which has the potential to enable vaccine manufacture of an increased range of strains (Berryman, Clark et al. 2013).

Groups working in Japan have recently begun to explore the possibility of using chimeric plant viruses engineered to contain short viral sequences as a safe way to produce vaccines. Li *et al* showed that a 9 amino acid sequence from FMDV VP1 could be expressed on the surface of apple latent spherical virus and produced in *Nicotiana benthamiana* plants. The same strategy applied to expressing epitopes from zucchini yellow mosaic virus induced specific antibody responses in immunized rabbits (Li, Yamagishi et al. 2014), and thus may warrant further attention.

Alongside novel methods of subunit vaccine production, advances in our understanding of how best to design such vaccines also hold promise. A study in mice has highlighted the importance of the conformation and valency of the epitopes used: following previous work showing that dendrimeric B cell/T cell fusion peptides induced superior B and T cell responses and better protection compared to their linear counterparts, this study went on to reveal that fewer copies of the B cell epitope could be at least as effective as more copies, and that the inclusion of an optimally oriented T cell epitope was important for immunogenicity (Blanco, Cubillos et al. 2013). In target species, we are some way from a parallel level of knowledge, but progress is being made, notably towards the definition of predicted CD8 T cell epitopes in swine (Liao, Lin et al. 2013), which as seen above may be a valuable addition to the vaccine-stimulated anti-FMDV response.

An interesting approach to vaccine design involves immunising with a recombinant live attenuated Peste des petits ruminants virus (PPRV) expressing the VP1 gene from FMDV. In goats this strategy successfully stimulated both neutralising antibodies to PPRV and enabled protection from virulent FMDV challenge after a single vaccination (Yin, Chen et al. 2014).

→ While unconventional, the potential economic advantages of a dual vaccine are undeniable, and this study highlights the power of using the PPRV as an internal live viral adjuvant which may serve to

elevate the anti-VP1 response to a protective level in sheep and goats, usually unattainable with conventional subunit vaccines.

### *Needle-free administration*

A major challenge in vaccine design is the move away from needle-based administration systems. Given the importance of inhalation as a route of transmission of FMDV, and the known importance of the local mucosal immune response for rapid protection, FMD is in fact a good candidate for an effective intra-nasal or even oral vaccine. Researchers working in Korea orally administered recombinant FMDV VP1 conjugated to Co1, a peptide mimic of bacterial cell wall components, which targets the protein conjugate to M cells of the intestinal Peyer's patches, the gateway to mucosal lymphoid tissue, to enhance its immune-stimulatory capacity. While protection assays were not included, an enhanced VP1-specific IgG and IgA response was evident in the mice, both in the mucosa and systemically, relative to VP1-alone treatment (Kim, Lee et al. 2013). Similarly, Iranian scientists have shown that the inclusion of inactivated FMDV into chitosan nanoparticles for intra-nasal application is effective in guinea pig models, particularly in terms of inducing high levels of mucosal IgA production (Tajdini, Amini et al. 2014). Further improvements might be made by targeting the chitosan nanoparticles to antigen-presenting cells within the mucosa via their mannose receptors: guinea pigs intra-nasally immunized with mannosylated chitosan nanoparticles containing a plasmid encoding FMDV VP1, mounted strong FMDV-specific IgA responses in the nasal mucosa and exhibited 60% protection from challenge (compared to 80% from conventional vaccine) (Nanda, Hajam et al. 2014).

→ Little work has been done on needle-free administration of mucosal-targeted vaccines in natural host species of FMDV. While any studies in non-natural target species must be interpreted cautiously, the strategies noted above are novel, rational and warrant serious consideration for evaluation and optimisation in susceptible livestock species.

### *Vaccine adjuvants*

The adjuvant field is appropriately an area of much active research, but it must be borne in mind that murine immune systems differ profoundly to those of target species, as does their response to the artificially forced FMDV challenge used to evaluate protection. As well as evaluation in target species and comparison with commercial vaccine, qualitative aspects of the induced response should be studied in order to understand their correlation with the speed, robustness and longevity of protection from challenge. Physiologically-relevant adjuvants should be preferred for this reason, as opposed to

synthetic/non-specific immune stimulants or those originating from non-viral pathogens, which risk sub-optimal polarization of the immune system.

The adjuvant research field has expanded dramatically in recent years with an increasing appreciation that the agents added to vaccines have not only the potential to magnify the immune response to the target antigen, but to shape the intricacies of the long-lived response to the immunizing agent. As seen above, the quality of the response can be just as important as the quantity when determining long-term protection from disease.

Several studies in mice have tested different adjuvants to FMDV vaccines and reported a level of success, though the extent to which this is translatable to natural FMD target species remains to be seen. Combining oral adjuvants with conventional FMDV vaccination is potentially attractive, and a study in mice fed a plant-derived polysaccharide from *Radix Cyathulae officinalis* Kuan and also given conventional FMDV vaccine, saw significantly higher FMDV-specific antibody titres, increased frequency of CD4<sup>+</sup> IL-2, IFN $\gamma$  and IL-4 producing cells, and decreased frequencies of T-regulatory cells compared to conventional FMDV vaccine-treated animals (Feng, Du et al. 2013). Similar results have been produced by the same group using different antigens in mice (Feng, Du et al. 2014). While no challenge data are available, the detailed characterization of the ensuing immune response to the vaccine is encouraging on a number of levels, and coupled with the advantages of oral administration of a natural product, warrants further investigation in appropriate animal species.

Using Toll-like Receptor (TLR) ligands as adjuvants to stimulate antigen-presenting cells is a popular approach, and there is some evidence that Poly I:C, a mimic of a virus-derived TLR3 ligand, can significantly increase cellular and humoral immune responses and reduce inter-individual variation in protection in pigs when combined with a multi-epitope subunit FMDV vaccine (Cao, Lu et al. 2013), supporting their earlier data in mice (Cao, Lu et al. 2012). TLR9 detects DNA patterns associated with microbes, including CpG oligodeoxynucleotides (ODN). ODNs are well-known immunostimulators, but a recent study showed the potential advantage of a novel conformation of ODN; the structure of the ODN determines the differential activity of the adjuvant on human immune cells, affecting levels of cellular internalization, intracellular targeting of the ODN and induction of Type I IFNs. The authors also combined nanorings of ODNs with inactivated FMDV vaccine and observed enhanced Th1 responses in mice (Gungor, Yagci et al. 2014). The nanoring concept is clearly distant from application to commercially available FMDV vaccines at this stage, but may prove a useful tool for understanding the impact of differential antigen targeting upon ensuing immune responses, and therefore to rational design of improved vaccines and adjuvants in the FMD field.

Many adjuvants will increase the magnitude of some aspect of the immune response, and may also increase the speed of induction, but the polarizing effects on the immune system are often overlooked and ill-understood. Using physiologically relevant adjuvants possesses the advantage that the immune response that's enhanced should also be of the "right" type to counter the viral threat. For example, the cytokine IL-2 may deserve investigation as an immune-stimulating addition to FMDV vaccines (Chen, Zeng et al. 2014), perhaps to increase the speed of onset of adaptive immunity in an outbreak situation. Of particular interest, one study assessed the potential of using small synthetic RNAs of the FMDV IRES as a novel adjuvant to two inactivated FMDV vaccine formulations in mice, and found that VNT were consistently higher in IRES-adjuvanted groups, that the onset of neutralising antibody was faster, and mean viraemia was lower following challenge (Borrego, Rodriguez-Pulido et al. 2013). In many ways this, or similar, strategies could represent the ultimate in rationally-designed adjuvants. Using a highly immune-stimulatory component of the live virus itself, in a non-infectious form, accompanied by inactivated FMDV protein shells should not only increase the magnitude of the immune response, but will go some way towards ensuring that the vaccine-induced response is a closer match to that induced by live virus, and needed for protection against the same threat.

Combining adjuvants aims to achieve a similar improvement to the tailoring of the response, and several publications have shown synergistic effects: both plant-derived adjuvants, in this case Rapeseed Oil and Ginseng Saponins (Zhang, Wang et al. 2014), and the synthetic TLR7/8 ligand R848 and poly I:C combined with traditional aluminium hydroxide-adjuvanted vaccine (Zhou, Li et al. 2014) have shown promise. Potential differences in receptor expression amongst immune cells and in the fundamental processes of immunity between inbred laboratory mice and outbred cattle, pigs or sheep, for example, render it impossible to predict the extent to which such results are translatable between species, though the approach is certainly of interest.

→ The adjuvant field is appropriately an area of much active research, but it must be borne in mind that murine immune systems differ profoundly to those of target species, as does their response to the artificially forced FMDV challenge used to evaluate protection. As well as evaluation in target species and comparison with commercial vaccine, qualitative aspects of the induced response should be studied in order to understand their correlation with the speed, robustness and longevity of protection from challenge. Physiologically-relevant adjuvants should be preferred for this reason, as opposed to synthetic/non-specific immune stimulants or those originating from non-viral pathogens, which risk sub-optimal polarization of the immune system.

*Differentiation of infected from vaccinated animals (DIVA)*

An obstacle to the use of vaccines during outbreaks in FMD-free countries is the subsequent inability to differentiate infected-recovered animals from vaccinates that also test positively for FMDV-specific antibodies. Conventional vaccines have traditionally relied on the absence of antibodies to viral NS proteins to enable differentiation of infected from vaccinated animals (DIVA), but this relies on every infected animal making the anti-NS response, and zero contamination of the vaccine with NS proteins. A marker vaccine with a deletion within FMDV 3A may offer some improvement (Li, Lu et al. 2014). However, this strategy similarly relies on 100% response rate of infected animals to the deleted region of 3A. The use of attenuated non-transmissible deletion mutants appears equally effective and far less risky in terms of accidental release (Uddowla, Hollister et al. 2012), similar to the newly-licensed US vaccine, further increasing the attractiveness of such approaches.

#### *Quality control and vaccine matching*

Methods for assessing the quality of FMD vaccines are well established and internationally accepted (European pharmacopoeia 2012, OIE 2015). However, there is always the potential to improve existing methods and research is ongoing.

A recent investigation of locally-produced and imported FMD vaccines available in Pakistan revealed alarming differences in quality (Jamal, Shah et al. 2013), clearly illustrating the importance and need for effective quality control in the vaccine-manufacturing process. However, advances in vaccine technology mean that state-of-the art methods for antigen production, purification and formulation may be technically and/or economically out-of-reach for some endemic countries. Scientists working at the Pirbright Institute have engineered a recombinant FMDV expressing the influenza haemagglutinin protein and a FLAG tag, either of which can be used to cheaply and easily purify virus from infected cell cultures (Seago, Jackson et al. 2012).

→ Coupled with appropriate quality control measures, improved methods enabling the rapid and effective purification of FMDV for vaccine production purposes could markedly improve control in endemic areas in a short time span and their development should be supported.

→ Routine independent evaluation of vaccine batch quality is fundamental to all successful vaccination programmes and needs to be promoted.

The quantity of intact viral capsid present in dose of vaccine is critical for immunity. Novel approaches have been developed to improve the measurement of capsid stability, something traditionally difficult to assess (Thermoflor assay, PaSTRy: Particle Stability Thermal Release Assay). As well as the assessment of vaccine quality, these methods will aid the study of determinants of capsid stability,



highly relevant to vaccine development (Walter, Ren et al. 2012, Seago 2014). Quantification of vaccine antigen content using specific Llama antibodies has also shown promise (Perez-Martin 2014).

An important measure of the quality of a vaccine is the vaccine potency test, which aims to measure protective power and ensure between-batch consistency. Traditional potency tests required direct challenge of a limited number of animals to measure the protection of a given vaccine or vaccine batch, but this approach is time-consuming, expensive, and may not predict protective power in the field (Goris, Merkelbach-Peters et al. 2007, Knight-Jones, Edmond et al. 2014). Alternative approaches continue to be explored and are used routinely in some regions: modifying the OIE challenge test procedures could make it possible to reduce the number of animals used with minimal losses of sensitivity or specificity (Reeve, Cox et al. 2011). Attempts to correlate bovine serological responses to vaccine protection have potential and are increasingly used for batch quality evaluation, murine responses may also be of use (Molin-Capeti, Sepulveda et al. 2013). The newly-licensed Ad5-vectored FMD vaccine is, by its nature, highly amenable to *in vitro* testing for batch potency and inter-batch consistency. In parallel with development of the vaccine, researchers at PIADC have defined a panel of tests including traditional modified-live vaccine virus infectivity assays, coupled with liquid chromatography and several immune-chemical assays, that may have the potential to replace live animal potency testing for this vaccine (Brake, McIlhaney et al. 2012).

Various *in vitro* methods of estimating cross-protection against heterologous strains have been proposed, but a recent study concluded that only the liquid-phase blocking ELISA offered sufficient reliability and accuracy to be a serious consideration (Tekleghiorghis, Weerdmeester et al. 2014). That said, *in vitro* methods proved valuable in understanding a lack, or loss, of vaccine mediated protection during outbreaks of divergent strains in Ecuador between 2008 and 2011 (Maradei, Perez Beascoechea et al. 2011, Maradei, Malirat et al. 2013). Furthermore there are data to suggest that *in vitro* matching using IgG subtypes and avidity tests may perform better than the traditional  $r_1$  value tests (Lavoria, Di-Giacomo et al. 2012, Brito, Perez et al. 2014); but while genetic sequencing should be able to predict antigenic sites, and therefore vaccine matching, a recent study using serotype A FMDV concluded that this approach was insufficiently reliable (Ludi, Horton et al. 2014).

Overall, a widespread move from large animal challenge experiments towards *in vivo* laboratory models or *in vitro* assays to predict vaccine potency is not imminent, particularly in the case of animals less well-studied than cattle, such as buffalo (Madhanmohan, Yuvaraj et al. 2014), or goats and sheep (Madhanmohan, Nagendrakumar et al. 2012), where live challenge remains the gold standard. However, further careful exploration of possibility of using small animal models, or of reduced numbers of large animals (Reeve, Blignaut et al. 2010, Reeve, Cox et al. 2011), and defining the most

appropriate surrogate measures for *in vitro* assay should move us closer toward this goal in the near future (Reeve, Blynaut et al. 2010, Reeve, Cox et al. 2011).

It has become apparent in recent years that while laboratory controlled infections of vaccinated animals are well-accepted, they are typically variable, under-powered (Goris, Merkelbach-Peters et al. 2007) and poorly recapitulate many aspects of field infections. Hence, there has been increasing interest in the evaluation of actual protection achieved under field conditions (Knight-Jones, Edmond et al. 2014). This is an important, but neglected, area as while different approaches have been investigated, further assessment of their strengths and weaknesses is required before a standardized approach can be adopted (Muleme, Barigye et al. 2012, Enekave, Li et al. 2013, Knight-Jones, Bulut et al. 2014). Again, the measurement of serological parameters has proven particularly useful when comparing the field efficacy of different vaccination schedules, in this case during the FMD outbreak in Korea in 2010/2011 (Lee, Lee et al. 2013).

### Research updates

The vaccine research and development field continues to be well investigated by numerous institutes around the world.

CEVAN (Centro de Virología Animal) of Argentina is exploring the use of novel vaccine strategies incorporating replicative and non-replicative vectors in combination with cytokines, viral replicons or recombinant bacterial phages to deliver FMDV immunogens.

Researchers working in China at the LVRI are collaborating with scientists at CODA in Belgium and the Pirbright Institute in the UK to understand optimal induction and measurement of mucosal immunity against FMDV in cattle. Using nanoparticles to deliver FMDV antigen and cytokine combinations showed potential to induce early nasal IgA responses, the magnitude of which was inversely associated with disease severity, virus excretion, and onset of clinical symptoms. Alongside, LVRI scientists are developing and testing novel peptide epitope vaccines, synthetic peptide vaccines, inactivated engineered FMDV vaccines, and a vaccine based on a recombinant baculovirus expressing the whole FMDV capsid. In addition, using *E. coli* to produce FMDV VLP was effective and is being further explored as a vaccine production strategy.

In Belgium, CODA has been coordinating the FMD-DISCONVAC (development, enhancement and complementation of animal-sparing, foot-and-mouth disease vaccine-based control strategies for free and endemic regions) research program that has been running since 2009. Within the project remit,

CODA itself is currently focusing on understanding immune correlates of protection, developing *in vitro* antibody-based assays to measure vaccine purity, and improving methods of quantifying antigen payload in manufactured vaccine batches. In addition, a set of studies on correlating immunological measures with homologous and heterologous protection from challenge following vaccination have yielded important results. A refined method for predicting changes in r-values from sequence changes was proposed, which together with the experimental data has the potential to improve vaccine-matching predictions. Promising vaccine trials have also been conducted: both canine adenovirus- and encephalomyocarditis virus- vectored FMDV vaccines have been produced and tested, as well as replication defective human adenovirus- (with and without IFN $\alpha$ ) and Sendai virus- vectored vaccines that have been developed specifically to enhance the mucosal immune response.

The FLI have also been working towards the FMD-DISCONVAC project, carrying out several cross-protection studies using conventional monovalent serotype A vaccines. Overall, while challenge virus specific VNT titres correlated well with protection, they uncovered evidence that some recent isolates from the Middle East cannot be covered by the high potency A vaccines that previously did provide sufficiently broad cross-protection. In addition, the FLI is testing the potential of viral vectors expressing different combinations of FMDV proteins to be used as novel vaccine candidates, with animal challenge experiments planned for the most promising candidates.

The University of Glasgow is heavily involved in developing strategies to increase the effectiveness of vaccination against FMDV, with focus on India. In particular, they aim to: define improved tools for vaccine strain selection via reverse genetics and modelling approaches; to determine whether cross-serotype protective antibodies can be induced, and to characterise them; and to develop new approaches to monitor FMDV infections within cattle populations to trace virus circulation and permit measurement of vaccine effectiveness. In addition, the possibility of improving FMDV vaccine formulation using novel adjuvants developed for use in humans is currently being assessed, with emphasis on strengthening the T cell component of immunity to FMDV. Alongside improvements to vaccination/monitoring procedures and the vaccine formulation itself, researchers at Glasgow are also addressing ways of replacing live animal challenge with *in vitro* measures of vaccine efficacy. The current project aims to develop a framework to better understand the relationship between serological assays and live animal challenge results, exploiting modern statistical tools to enable accurate prediction of protection based on *in vitro* immunological parameters.

A collaborative project between INTA and the Biotechnology Research Institute of the National Research Council, Canada aims to develop a panel of VLP-based FMDV vaccines targeting locally-circulating strains for initial testing *in vitro* and in a mouse-based *in vivo* protection model. The most

promising candidates will move forward into immunological characterisation and challenge in cattle. INTA also has several other projects focusing on development of novel or improved vaccines, including extending their study of the use of dendrimeric T and B cell epitope peptides to induce immune responses from pigs into cattle, and evaluating safety and efficacy parameters of inactivated FMDV formulated with a novel vaccine adjuvant, Providean-AVEC®, developed by INTA scientists with the assistance of the National Scientific and Technical Research Council of Argentina. This nanoparticle-based adjuvant has been rationally designed and targets host antigen-presenting cells with TLR ligands; initial studies in mice, cattle, pigs and fish using a range of antigens have indicated superior performance to either oil- or aluminium hydroxide- based adjuvants. A second adjuvanting approach being trialled is the use of recombinant baculoviruses combined with conventional FMD vaccines. The current project aims to characterise the effect of including baculovirus as a means to stimulate both innate and adaptive responses in mice, and will define immune response profiles and effect on protection against challenge. DNA vaccines are low-cost and safe, but often of insufficient potency for use in the field. In a planned study, a DNA vaccine encoding the FMDV polyprotein will be co-administered to mice with plasmids containing the sequence for immune-stimulatory CD40L and IL-15, either with or without an additional adjuvant, to understand the potential of this strategy to improve immune responses and protection from virulent FMDV challenge. Lastly, while many conventional serological assays have been developed for cattle, their performance in other species is less well validated. INTA plan to assess the suitability of these assays for use in buffalos and pigs, in order to identify the most appropriate high throughput immunological assay to measure anti-FMDV immune responses following vaccination or infection of these species.

At the Indian Veterinary Research Institute (IVRI), ongoing work is focused on the use of a baculovirus platform to develop a novel vaccine and accompanying NSP-based diagnostics to enable DIVA. The use of the bacterial TLR ligand, flagellin, is also being explored as an adjuvant, as is the inclusion of recombinant Hsp60, which functions as a chaperone protein that may improve vaccine antigen presentation on host antigen-presenting cells, and therefore increase immunogenicity. Guinea pig data appear promising, and trials in cattle are planned. To complement this work, a collaborative project between the IVRI, the FMD project directorate in Mukteswar, India, and PIADC is being undertaken to develop and test an adenovirus-based vaccine encoding the capsid sequences of Indian vaccine strains of FMDV. Preliminary cattle data support the immunogenicity of the vaccines, but indicate the need for further dose/schedule optimisation before the initiation of planned challenge studies.

The National Livestock Resources Research Institute (NaLIRRI) in Uganda is planning a set of studies defining important cellular and humoral immune parameters in the response to conventional vaccine across a range of relevant species, including cattle, goats, sheep and pigs. In particular they will focus on the impact of age and breed in determining the magnitude and quality of response.

In South Africa, the OVI is involved in a multi-site project with the University of Glasgow, The Pirbright Institute and SACIDS (Southern African Centre for Infectious Disease), aiming to develop improved indirect and informatics-based methods of vaccine matching with field strains. This interesting approach uses mathematical predictions of protective B cell epitopes, based on genetic and structural data and *in vitro* assays, with which to compare vaccine and field viruses and thereby predict the extent of shared antigenicity. Associated work has also highlighted qualitative differences between assays often used interchangeably to determine antigenic match between field viruses and vaccine strains, which is an unresolved issue for effective vaccine strain selection. An approach to overcome the impact of viral antigenic drift is also being developed in conjunction with PIADC, which may enable molecular “fine tuning” of vaccine strains to improve their protective power in the field. A second project aimed at improving current vaccine production strategies is also underway, in collaboration with Wellcome Trust Consortium scientists, in Oxford, UK. Through structural modifications to the virus, substantial improvements in thermal stability and cell-culture adaptation have been made, which may overcome pressing problems in the conventional manufacturing process, particularly for SAT serotype FMDV. Planned trials in cattle will assess immunogenicity, efficacy and shelf life of the newly-engineered vaccines.

The Pirbright Institute plan to employ reverse genetics approaches (capsid switching, partial capsid switching and site-directed mutagenesis), to understand the relationship between serological cross-reactivity, capsid amino acid sequence conservation and capsid structure. They will trial the strategy of combining heterologous serotype A vaccine strains in an attempt to improve antigenic cover against a third heterologous strain, in conjunction with Indian Immunologicals Ltd (IIL) in Hyderabad.

PIADC is involved in a suite of collaborative and independent research projects aimed at improving understanding of the vaccinal immune response, the development of novel vaccines, and approaches to overcome the limitations of conventional vaccines/their production. With the University of Vermont, Canada, the impact of breed-specific responses to FMDV are being incorporated into a strategy to accelerate the development of improved vaccines, and alongside, data are being collected on the impact of bovine leucocyte antigen sub-type on responses to the human adenovirus-vectored FMD vaccine developed by PIADC. With scientists at Australia’s CSIRO-AAHL, PIADC aims to

understand the optimal vaccination protocols for sheep, and to define the appropriate challenge conditions with which to test emergency vaccines in this species.

In a separate development, ARS scientists at the PIADC have engineered a recombinant vaccine seed virus (the FMD-LL3B3D platform) to improve safety in case of virus escape from the production facility. This virus can replicate in cell culture but is attenuated in cattle and swine; moreover the deletion of selected epitopes from the virus should allow the development of companion tests enabling DIVA. Importantly, relevant capsid sequences can be substituted into the chimeric virus seed to provide immunity against different strains. This engineered vaccine seed is then used to produce inactivated vaccine in the conventional way. This new vaccine technology has been transferred to Zoetis and is currently under development with the support of ARS scientists.

→ Chimeric vaccines, such as the FMD-LL3B3D, represent another promising avenue for the production of improved FMD vaccines.

The University of Wageningen in the Netherlands is conducting a set of studies that are linking the antibody response and protection induced by conventional FMD vaccines with vaccine dose and antigen concentration, as well as mode of administration. The sensitivity of response to the composition of the vaccine in particular has highlighted the risk of generalising results between vaccines from different manufacturers, the impact of which has been under-estimated until now. They are also concerned with developing reliable *in vitro* assays that correlate immune parameters (such as serology, measurement of IFN- $\gamma$  release, capacity of sera to promote FcR-mediated FMDV phagocytosis and the plasmacytoid DC IFN- $\alpha$  response) with *in vivo* protection, as a means to reduce reliance on animal experiments. In parallel, investigations are being made into methods to improve vaccine production and quality monitoring and thereby increase overall inter-batch confidence and industry standardisation.

IZLER scientists are similarly aiming to improve vaccine production and quality control by the development of novel ELISAs for quantification of FMDV 146S or 12S. Moreover, in collaboration with CODA in Belgium, they are conducting work to standardise serological testing of field samples between different laboratories and to understand the extent and impact of existing variations.

For a summary of research updates from contributing institutions, see appended Table 2.

## Research priorities

The use of FMD virus-like particles as a vaccine has the potential to revolutionise the production of effective FMDV vaccines in a safe and cost-effective way. However, their efficacy in species other than cattle is incompletely defined, as are their effects on non-humoral aspects of the immune response.

Several novel adjuvanting or vectoring strategies for various FMD vaccines also show promise but will require further testing in target species under physiological challenge conditions. Whilst convenient and cheap, the use of murine systems may need to be de-emphasised in light of accumulating data on relevant differences between their immune systems and those of natural FMD target species. In addition, the importance of qualitative aspects of the response to FMDV infection and vaccination, such as the characteristics of non-neutralising antibodies, or the immunoglobulin isotype ratio induced, must be recognized, and these parameters should be included during the evaluation of novel adjuvants or vaccines. To provide meaningful reference, conventional vaccine should be included alongside any novel approach in all studies.

Improved methods enabling the rapid and effective purification of FMDV for conventional vaccine production are under development which, if rolled out widely, could improve control in endemic areas within a relatively short time.

Some studies have begun to indicate possible ways of using needle-free strategies to induce protective mucosal responses to FMDV vaccines, but further study of mucosal immunity in target species (perhaps requiring the development of additional tools), both in general and in relation to FMD, will be required to realise the potential of this strategy.

Despite much effort, reliable immune correlates of protection remain to be identified. An absence of standardised approaches to vaccination and challenge is likely to have contributed to this problem, and should be addressed as a matter of urgency, ideally in collaboration with the OIE, for example, to ensure widespread uptake of recommendations in future studies.

Predicting vaccine matching with emerging field strains remains a challenge.

Control of FMD in endemic regions would be markedly assisted by improving formulation of quality controlled vaccines and reagents to extend shelf life and reduce cost, thereby improving availability and efficacy.

Lack of independent evaluation of vaccine quality is a major deficiency that holds back many control programmes.

## Biotherapeutics

### From the 2010 Gap Analysis

Priority research aims stated were to:

1. Testing Ad5-IFN distribution and expression in cattle after aerosol exposure
2. Evaluate the ability of Ad5-type I IFN platform to confer rapid onset of protection (18 hr) against several FMD serotypes and subtypes

### Literature review

Vaccines as a primary line of defence suffer from the unavoidable weakness that the vertebrate adaptive immune system needs time, at least 7 days from the first inoculation, in order to mount a potentially protective antibody and/or T cell response. In an outbreak situation, this gap may be bridged by the application of rapid, short-acting biotherapeutics aiming either to stimulate a non-specific anti-viral state in the animal, or to specifically inhibit a part of the viral life cycle for sufficient time to allow a vaccine to become available, or until the response to the vaccine grows sufficiently strong.

#### *Immune-modulators*

Replication-defective human adenovirus 5 (Ad5) has attracted much interest as a potential vector for bovine and porcine interferons to improve early control of FMDV. Earlier data showing protection of swine from multiple serotypes of FMDV as early as 1 day post-administration of the Ad5 bearing porcine IFN  $\alpha$  (Dias, Moraes et al. 2011) have been supported and extended to describe the ability of Ad5 encoding Type III IFN to either prevent or substantially protect from FMDV in both pigs (Perez-Martin, Diaz-San Segundo et al. 2014) and cattle (Perez-Martin, Weiss et al. 2012). The lack of protection of cattle by Ad5 encoding bovine Type I IFN is perhaps consistent with the finding that only low levels of IFN $\alpha$  are seen in this species during FMDV infection (Windsor, Carr et al. 2011), which remains to be fully understood in terms of species-specific mechanisms of immunity.

Though effective, impractically high doses of the Ad5-vectored interferons are likely to be required for their direct applicability to outbreak control in the field. Accordingly, several modifications to the approach have been attempted to increase potency; instead of incorporating the sequence for the interferon itself into Ad5, encoding a constitutively-active transcription factor targeting type I IFN induced high levels of IFN $\alpha$  production in mice, and protection from FMDV challenge as soon as 6



hours post-treatment, which lasted for approximately 4 days (Ramirez-Carvajal, Diaz-San Segundo et al. 2014). Treatment of pigs with stabilised poly I:C combined with replication-defective adenovirus encoding IFN $\alpha$  was also effective in inducing sterile immunity to A serotype FMDV, and at high doses the stabilized poly I:C alone could stimulate high levels of Type I IFN production *in vivo* and protect from disease (Dias, Moraes et al. 2012). Data in mice indicate that including IFN $\gamma$  in tandem with IFN $\alpha$  in the recombinant adenoviral vector, and exploiting FMDV 2A to cleave the two cytokines after transcription, might be more effective in preventing FMD than either protein singly (Kim, Kim et al. 2014), which would be easily tested in swine. Taking a different route, exchanging the Ad5 vector for Venezuelan equine encephalitis virus empty replicon particles encoding porcine IFN $\alpha$  protects porcine cell lines from infection by FMDV, as well as rendering mice resistant to lethal challenge 24 hours post-administration (Diaz-San Segundo, Dias et al. 2013).

An alternative interferon-related target is interferon-induced transmembrane protein 3 (IFITM3), which is an antiviral effector of the innate immune system that has been recently characterised in swine. Inducing expression of sIFITM3 in BHK cells blocked FMDV infection at the point of entry, and sub-cutaneous inoculation of suckling mice with a plasmid encoding sIFITM3 was able to protect against lethal challenge at 48 hours post-administration (Xu, Qian et al. 2014). Direct treatment with small synthetic RNA sequences from the FMDV IRES or 3' non-coding region of FMDV at the same time as pathogen inoculation was also able to protect suckling mice from lethal challenge with Rift Valley Fever Virus (Lorenzo, Rodriguez-Pulido et al. 2014), highlighting the potential utility of the strategy using either FMDV or unrelated viral sequences, which is yet to be tested as a biotherapeutic in FMDV infection

While the potential benefits of biotherapeutics are clear, their integration into epidemic control strategies in the field remains untested. As immune-modulators, they have the potential to both positively and negatively impact immune responses to pathogens: to illustrate, there is evidence from swine and mice that high levels of type I IFN early in viral infection may be involved in suppressing the T cell response (Summerfield, Alves et al. 2006, Langellotti, Quattrocchi et al. 2012) and therefore it cannot be assumed that the application of IFN $\alpha$ -based biotherapeutics, prior to, or concurrent with a conventional vaccine will not adversely impact the response to that vaccine.

Previous work at PIADC ago that showed that porcine IFN- $\alpha$  enhanced protective immune responses to FMDV in pigs vaccinated with a replication-defective adenovirus containing FMDV capsid and 3C proteinase coding regions (Ad5-A24) (de Avila Botton, Brum et al. 2006).

→ The interaction of immune-modulating biotherapeutics with the ensuing proposed vaccine must be addressed as a matter of urgency, with particular emphasis on detailed characterisation of both qualitative and quantitative effects on the quality and duration of the immune response in key target species.

#### *Anti-viral interventions*

Non-immune-modulating biotherapeutics have been less explored, but may also hold promise. Harnessing the host's own transcriptional silencing mechanisms to prevent FMDV genome replication has been attempted with limited success using short-interfering RNAs, but micro-RNAs may hold more promise. A plasmid encoding dual miRNAs targeting the IRES region proved able to delay or prevent death in suckling mice when co-administered with high doses of FMDV from multiple serotypes. (Chang, Dou et al. 2014).

Moreover, the many and varied roles of FMDV 3Cpro make it an attractive target for pharmacological inhibition, and a recent study both designed an improved cell-based assay to test candidate compounds and used it to identify AG7088 (Rupintrivir) as a potent 3C inhibitor (van der Linden, Ulferts et al. 2014). This is particularly interesting as Rupintrivir was originally developed by Pfizer as an aerosolised treatment for the common cold in humans, and therefore may prove amenable to further development as an intra-nasal biotherapeutic to impede the early stages of FMDV infection in outbreak situations.

### **Research updates**

CODA has a program of research targeting discovery of anti-FMDV biotherapeutics with which to supplement current outbreak control measures. They have found three different classes of anti-viral compounds are active against FMDV in both guinea pigs and immunodeficient mice. One compound underwent several rounds of modification and retesting that produced lead compounds active against the FMDV protease 2C, and inhibited the early phases of replication of the virus. However, technical limitations have precluded further development of these compounds for large-scale use. In contrast, high-throughput screening of 65,000 small molecules revealed three compound classes active against multiple serotypes of FMDV *in vitro* that could be easily and cheaply manufactured on large scales. Preliminary studies in guinea pigs and mice are ongoing.

The IVRI are interested in the potential use of small interfering RNA (siRNA) as an antiviral strategy applicable to FMDV control. Four siRNA sequences targeting FMDV 3Cpro have been designed and

show efficacy in reducing FMDV replication *in vitro*. Alongside, IVRI plan to exploit the adenoviral delivery system to develop and test IFN  $\alpha$  and  $\lambda$  based therapeutics for early FMD control.

INTA are also interested in the possibility of using RNA interference (RNAi)-based antivirals to control FMDV, but with a focus on artificial microRNAs, which may offer a safer means of intervention than the use of short-hairpin RNAs or small-interfering RNAs which have previously been proven effective against FMDV replication in culture.

Scientists working at PIADC are focusing their biotherapeutics research on immune-modulators, particularly those able to induce NK cell activity, such as IL-15. However, as little is known of the precise role of NK cells during FMDV infection of cattle or pigs, work will be conducted to characterise the function of NK cells in the early stages of infection of these species. New reagents will be generated to assist this work, and biotherapeutics will undergo testing both *in vitro* and *in vivo*.

For a summary of research updates from contributing institutions, see appended Table 2.

### Research priorities

In 2010, the use of adenovirus to vector IFN $\alpha$  (Ad5-IFN $\alpha$ ) into hosts and induce short-lived non-specific protection from FMDV appeared promising. It now seems that Ad5-IFN $\alpha$  alone is insufficiently potent for use in outbreak situations. However, studies combining Ad5-IFN $\alpha$  with an additional immune stimulator reveal some potential, as does Ad5 encoding an IFN-stimulating transcription factor in place of IFN $\alpha$ . Substituting Type III IFN into the Ad5 vector appears more effective, particularly in cattle, and warrants further investigation.

An area of urgent unmet research need is the potential interaction of immune-modulating biotherapeutics with the response to the ensuing proposed vaccine. Before their use in the field, we must know how expression of large amounts of these innate immune cytokines affects both qualitative and quantitative aspects of the immune response in target species. For example, high levels of IFN $\alpha$  can adversely impact the magnitude of T cell responses, which in the case of FMD vaccination, could preclude the formation of sufficient immunity to confer protection from disease.

Non immunological, small molecule inhibitors of FMDV 3Cpro have been identified, but remain to be tested in target species *in vivo*.

RNA interference-based strategies are also being explored, though their suitability for upscaling to the levels required in outbreak situations has yet to be assessed. More generally, the integration of biotherapeutics into existing emergency measures similarly remains to be achieved.

## **Disinfectants**

### **From the 2010 Gap Analysis**

Priority research aims stated were to develop low cost commercially available disinfectants for use in the inactivation of FMDV on contaminated surfaces found in farm settings and other susceptible environments.

### **Literature review**

Little has been published in this area in recent years, though a single study addressed the environmental and public health impacts of the mass usage of disinfectant during outbreaks of FMD in South Korea (Kim, Shim et al. 2013).

### **Research updates**

PIADC are leading current work in the disinfectant field. They have several ongoing and planned projects aiming to develop accepted disinfectant efficacy test methods and to establish the efficacy of selected antimicrobial pesticides for inactivating FMDV on a range of surfaces and under various conditions. In addition, IZSLER are investigating the kinetics of and conditions for chemical and thermal inactivation of FMDV in field and laboratory environments. The Pirbright Institute has an established quality-assured disinfectant testing service, and reference to the standards used here may prove helpful to the work of PIADC and IZSLER.

For a summary of research updates from contributing institutions, see appended Table 2.

### **Research priorities**

Planned and ongoing research would appear likely to meet the 2010 Gap Analysis goals. The potential environmental and public health impacts of disinfecting agents, whether novel or established, should be evaluated and incorporated into the decision making process on their use.

## Diagnosics

### From the 2010 Gap Analysis

Priority research aims stated were to:

1. Determine the link between molecular serotyping and protective immunity
2. Support the development of new technologies for pen-side testing
3. Evaluate and validate commercially available pen-side tests to “fit for purpose” for surveillance, response, and recovery
4. Proof-of-concept testing of herd immunity test correlated with efficacy of vaccine in the National Veterinary Stockpile
5. Identify FMDV-specific non-structural protein antigenic determinants for development of DIVA diagnostic tests
6. Develop serotype-specific rRT-PCR assay(s)
7. Development of TIGR technology for FMD serotyping/subtyping for rapid vaccine matching and monitoring variation of the virus during an outbreak of FMD
8. Assess the feasibility of infrared thermography as an FMD screening tool under different environmental field conditions in healthy and diseased animal populations. Assess the potential application of this technology to aid in the identification and sampling of suspected animals for confirmatory diagnostic testing
9. Investigate the use of artificial intelligence for the development of algorithms to recognize FMD signatures in domestic animal species (cattle, pigs)
10. Assess the use of air sampling technologies and validate their use for FMDV aerosol detection in open and enclosed spaces

### Literature review

Diagnostic tests are routinely used to confirm clinical diagnosis when outbreaks are detected; detailed analysis of the infecting isolate is important to allow routes of spread to be identified and appropriate vaccines to be selected, while serology is used to both establish freedom from infection and to monitor the likely level of post-vaccination immunity. The literature since June 2011 describes both novel diagnostic tools and refinements to existing methods or their use in various settings. In particular, the application of different diagnostic approaches to outbreak scenarios is a key factor, as there is likely

to be a sudden need to conduct large numbers of tests on critically short timescales (Paton and King 2013).

#### *Antigen detection*

To facilitate strain typing, RT-PCR methods have been developed that can rapidly amplify the capsid-coding region of the FMDV genome (Le, Lee et al. 2012, Xu, Hurtle et al. 2013); another novel RT-PCR used minor groove binder technology for the detection of FMD of all serotypes (McKillen, McMenemy et al. 2011). The Pirbright Institute have recently reported the development of serotype specific rRT-PCR assays for FMDV strains circulating in the Middle East (Reid, Mioulet et al. 2014), which was highlighted as a research priority in the 2010 GFRA Gap Analysis.

A number of RNA detection assays targeting the FMDV genome have been developed using reverse transcription loop-mediated isothermal amplification (RT-LAMP), with some detecting single serotypes and others multiple (Chen, Zhang et al. 2011, Madhanmohan, Nagendrakumar et al. 2013, Yamazaki, Mioulet et al. 2013, Ding, Zhou et al. 2014, Kasanga, Yamazaki et al. 2014). RT-LAMP was faster, simpler, more cost-effective and at least as sensitive and specific as RT-PCR. Furthermore, it has been successfully used in a portable platform to allow rapid diagnosis in the field (Abd El Wahed, El-Deeb et al. 2013).

→The development of RT-LAMP represents an important breakthrough that will enable increased usage and access to molecular diagnostics.

A number of studies describe the generation of monoclonal or polyclonal antibodies for use in FMD immuno-assays (Lin, Li et al. 2011, Chen, Peng et al. 2012, Cho, Jo et al. 2012). One paper described FMDV antigen detection using a novel direct ELISA that outperformed the conventional indirect ELISA (Morioka, Fukai et al. 2014). Superior serotype specificity was also obtained in a sandwich ELISA using recombinant integrin  $\alpha\beta 6$  as a capture ligand and serotype-specific monoclonal antibodies as detecting reagents (Ferris, Grazioli et al. 2011). Another study developed a recombinant single chain variable fragment as an alternative to immunoglobulins and applied it as a detection agent in an ELISA (Sridevi, Shukra et al. 2014).

#### *Antibody detection*

Conventional tests for antibodies recognizing FMDV structural proteins, which are raised in abundance after vaccination or infection, use detection antigens derived from live virus which requires specialized bio-safety level 3 facilities to produce safely. The use of recombinant technology as an alternative

source of test antigens showed promise in several studies (Ko, Lee et al. 2012, Basagoudanavar, Hosamani et al. 2013, Wong, Sieo et al. 2013). One study supported the use of the solid phase competition ELISA for FMDV structural protein antibody detection (Li, Swabey et al. 2012). Several publications described the development of in-house tests for the detection of viral NSP-specific antibodies, the basis of most DIVA assays (Jaworski, Compaired et al. 2011, Gao, Zhang et al. 2012, Sharma, Mohapatra et al. 2012, Srisombundit, Tungthumnyom et al. 2013, Biswal, Jena et al. 2014, Mohapatra, Mohapatra et al. 2014), including a Luminex assay (Chen, Lee et al. 2013). But regardless of the test used, relying on the detection of anti-NSP antibodies to detect infection in vaccinated populations is imperfect, as vaccinated cattle may occasionally sero-convert, particularly after repeated vaccination and persistently infected cattle with localised rather than systemic infection may not develop a detectable NSP antibody. Thus certifying freedom in vaccinated populations based on NSP sero-surveillance comes with an element of uncertainty.

### *Sequencing*

Next generation sequencing technologies have emerged that allow sequencing of not only the predominant sequence but also minority variants present within a single sample. This technology has been used to explore within-host virus evolution and selection in a way not previously possible (Wright, Morelli et al. 2011, King, Madi et al. 2012), substantially advancing our knowledge of FMDV population dynamics within the animal.

→ Molecular and genetic technologies play a central role in our ever expanding knowledge of virus ecology and transmission.

### *Pen-side tests*

Besides RT-LAMP, mentioned above, developments in pen-side diagnostics include the evaluation of a portable real-time PCR amplification platform (Madi, Hamilton et al. 2012). Another study described a high-speed quantitative PCR assay which provided results in <30mins (Wernike, Beer et al. 2013), which could be a substantial advantage in the urgency of an outbreak situation. Variations of the lateral flow device were produced, including one for the detection of vesicular stomatitis (Lin, Shao et al. 2011, Ferris, Clavijo et al. 2012, Yang, Goolia et al. 2013). The use of thermal imaging to assess hoof temperature for the early detection of FMD was found to be of limited accuracy (Gloster, Ebert et al. 2011).

→ Progress has been made in the field of rapid diagnostics, but further advances would be beneficial, particularly for methods that allow strain typing.



### *Screening*

A multiplex RT-PCR assay, combined with microarray typing, was used to detect and type all serotypes of FMDV and vesicular stomatitis virus in one test protocol (Lung, Fisher et al. 2011). Another multiplex PCR assay was able to screen samples for six different porcine pathogens that can present with similar clinical signs [including FMD] (Wernike, Hoffmann et al. 2013).

### *Sampling*

The use of cotton ropes as a means of obtaining oral fluid samples from pigs was found to have reasonable sensitivity (Vosloo, Morris et al. 2013). Similar results were found for rope-in-a-bait as of humane way of getting saliva samples from wild boar (Mouchantat, Haas et al. 2014). These approaches could be employed to address both feasibility and welfare considerations in regular sampling protocols, particularly those involving wildlife. An evaluation of oral swabs versus probang sampling found that early viraemia could be detected by both sampling methods in pigs and cattle, though persistent infection in FMDV carrier cattle could only be detected using probang samples (Stenfeldt, Lohse et al. 2013). Furthermore tonsil swabbing seems to be high effective at isolating virus in persistently infected African buffalo (Juleff, unpublished).

### *Broader issues*

A clear global picture of the distributions and movements of FMD strains within and between regions and countries is lacking. This is due to limited diagnostic capacity and sampling in many endemic countries (Namatovu, Wekesa et al. 2013). Cost and complexity present a barrier for the wider use of many of the diagnostic techniques mentioned above in developing countries: although there is no immediate solution to this problem, pen-side devices have proved useful, RT-LAMP promises to make molecular diagnostics simpler and more economical, and there is the prospect that sequencing technologies will also likely become more affordable in the future (King, Madi et al. 2012). In FMD-free countries, early detection of infection is critical and further developments in pen-side tests and pre-clinical diagnostics are needed (Paton and King 2013).

## **Research updates**

The Pirbright Institute is active in several areas of FMD diagnostic development, many mentioned in the above literature review (pen-side PCR, RT-LAMP, next generation sequencing, etc...). In collaboration with IZSLER, researchers are working on the development of new monoclonal antibody based assays for use in commercial tests. Cost, complexity and biosafety requirements of current tests

(antibody and antigen) present a barrier to some countries wishing to perform more FMD diagnostics. LVRI in China have a similar diagnostic research interest to The Pirbright Institute, and have developed two rapid pen-side tests in the form of a NSP immunochromatographic strip and a Dot-plot kit.

CODA is also working on ELISA tests for laboratories with limited facilities. In addition, within the FMD-DISCONVAC project, they have performed validation studies on the IgA and NSP ELISAs as DIVA tests and worked on Luminex assays. Like other laboratories they are active in twinning programmes to assist laboratories in endemic countries.

In Argentina, both INTA and CEVAN have done research to improve the use of serology in estimation of vaccine protection and immunity, as well as for detection of infection. When mass vaccination is used but disease is not present, field evaluation of vaccine protection has to rely on immune correlates and not natural challenge.

In Australia, CSIRO-AAHL has worked on diagnostic capacity in readiness of an FMD incursion. In New Zealand, AHL-NZ (Animal Health Laboratory – New Zealand) have performed evaluation studies for FMD diagnostics in red deer, which are farmed in large numbers and could play an important role should an outbreak occur.

The University of Glasgow's work includes the use of milk for NSP tests, PCR and virus sequencing to assess population burden of infection. In addition they are interested in assessment of vaccine effectiveness in the field and the development of diagnostics in East Africa. FLI have looked into non-invasive sampling methods, such as baited swab sampling of wild boar and air sampling for pre-clinical detection in cattle. They have also worked on various aspects aiming to optimise FMD diagnostics, including virus inactivation by nucleic acid extraction buffers.

The IVRI has looked at the use of a baculovirus expression system to produce the 3ABC protein for use in NSP tests without the need for live virus. Wageningen have also looked at NSP tests, assessing alternatives to the current commercial tests and studying the timing of NSP antibody responses after infection or vaccination in cattle and small ruminants.

Although initial findings at The Pirbright Institute indicated that the use of thermography for early FMD detection may not be promising, further evaluation of the technology continues at other sites, including PIADC. Plum Island have also developed a multiplex RT-LAMP that can simultaneously test and screen for FMD and Vesicular Stomatitis. In addition they are trying to produce inexpensive diagnostic kits for sub-Saharan Africa.

For a summary of research updates from contributing institutions, see appended Table 2.

### Research priorities

Continued development of molecular and genetic technologies combined with novel analytical techniques is vital, and will in turn benefit many different areas of FMD research.

Pen-side tests facilitate rapid outbreak detection, which is crucial for minimising the impact of an outbreak. Pen-side tests also allow FMD diagnostics where well-equipped laboratories are not easily accessible. Although progress has been made in the development of pen-side tests, ongoing research is required. In addition, the development of simple and affordable diagnostics, particularly for SAT viruses, for use in under-resourced laboratories should be considered a priority.

Although NSP serology is widely used, it has limitations and improved DIVA tests are urgently required. The same is true for vaccine matching assays. Relevant to this is the need to better understand the link between molecular characteristics of viruses and cross-protective immunity. Similarly, approaches and methodologies for assessing post-vaccination population immunity need to be strengthened, standardised and their use widely promoted.

## Understanding the Virus

### Pathogenesis

#### From the 2010 Gap Analysis

Priority research aims stated were to:

1. Identify determinants of viral virulence for different serotypes of FMDV in cattle, sheep, and swine
2. Investigate virus-host interactions at the primary sites of infection in ruminants and their role in determining infection
3. Determine the early events in FMDV pathogenesis in swine and small ruminants (i.e., primary site of replication, mechanisms of spread)

### Literature review

#### *Early events and their relation to virulence*

In part, our relative lack of understanding of the events occurring during and immediately following the host's initial exposure to the virus reflects the ongoing need for reliable and reproducible methods to simulate natural infection under laboratory conditions. In pigs, two simulated-natural inoculation systems were recently developed and tested: intra-oropharyngeal inoculation gave consistent and synchronous infections and was preferred over intra-nasopharyngeal administration, which was more variable. The same study also highlighted the likely importance of the oropharyngeal tonsils in early infection of swine (Stenfeldt, Pacheco et al. 2014). In cattle, administering FMDV-A24 either into the tongue epithelium or by aerosol, confirmed data from O serotype viruses that initial replication occurs in the nasopharynx, followed by expansion in the lung and systemic viraemia, as well as identifying a local Type I and III IFN response at the primary sites of infection. Comparing an attenuated mutant of the same strain revealed that virulence determinants at the point of infection were: virus-intrinsic factors, host defence mechanisms, and route of exposure (Arzt, Pacheco et al. 2014).

→ Widespread adoption of more physiologically relevant routes of infection for laboratory studies appears feasible and desirable to produce data readily translatable to field situations.

On the cellular scale, it has been known for some time that FMDV tissue tropism cannot be fully accounted for by receptor expression alone. Whole genome gene expression profiling now suggests

that a combination of receptor ( $\alpha\text{V}\beta\text{6}$  integrin) expression and availability, the capacity of the tissue to clear infected cells, and the extent of the type I IFN response locally are the key influencers of tissue tropism and the pathogenesis of the viral lesions (Zhu, Arzt et al. 2013). Understanding the tropism of FMDV for the heart muscle is particularly pressing, as myocardial infection is the most commonly identified cause of death in animals with FMD. New data from pigs infected with chimeric FMDV found that alterations to the capsid proteins VP1, VP2 and VP3, had the potential to markedly increase the proportion of infected animals dying from myocardial infection (Lohse, Jackson et al. 2012). A comparative analysis of fatal FMDV-associated myocardial inflammation in two pigs indicated that viral strain differences may also play a role in determining the precise pathology of the condition, but that regardless of strain, an abundant NK cell infiltrate was present within the heart muscle (Stenfeldt, Pacheco et al. 2014).

→ Moving forward, it would be of interest to determine which features of the strains are associated with increased fatality, perhaps focusing initially on capsid proteins, as well as investigating the properties of the NK cells infiltrating the heart, and whether they are part of the problem or the solution.

#### *Persistent infection*

Full understanding of the carrier state is a pre-requisite for rational development of vaccines able to induce sterile immunity and for planning safe and effective means to trade with endemic countries. The presence of FMDV in the germinal centres of carrier ruminants was described for the first time in 2012 by Juleff et al, working at the Pirbright Institute. Unexpectedly, this virus is also detectable in animals defined as non-carriers according to traditional methods (Juleff et al., 2012). Moreover, recent evidence indicates a similar phenomenon may occur in pigs (Stenfeldt, Pacheco et al. 2014), which is surprising as swine are considered non-carrier species.

→ These findings revolutionise our appreciation of FMDV persistence, but it remains to be seen whether their primary importance is as a depot of live virus leading to replication and the emergence of the classical “carrier state” or rather as a protective immunological resource with which to maintain high antibody titres for prolonged periods.

A useful tool for understanding persistence at the cellular level has been developed by researchers at the University of Wuhan in China. They established a persistently infected cell line and found that the molecular basis of persistence was based on selection for modifications to the cellular genome that enables resistance to the lytic effects of FMDV (Zhang, Li et al. 2013)

→ While *in vivo* persistence is unlikely to be mediated by the same mechanisms, this study offers a novel perspective on the problem and could be used as a starting point for investigations of cellular factors involved in pathogenesis and as a way to understand how to block FMDV lysis of target cells and therefore prevent spread of infection within the animal.

## Research updates

### *Persistence*

In an EuFMD-funded project, researchers at the National Agency for Food, Environmental and Occupational Health Safety (ANSES) in France plan to characterise both FMDV and host cell determinants of persistence on the cellular level, focusing on host transcriptional modifications that can overcome FMDV-mediated lysis. It will be interesting to compare the outcomes of this study with those already published (Zhang, Li et al. 2013) and to understand to what extent the findings are cell type/virus strain specific.

### *Transmission*

The Friedrich-Loeffler-Institute has generated important data during laboratory infections of wild boar, showing that the disease is clinically mild in this species but follows a similar time course to that in domestic pigs; no viral shedding was detected past 9 days post-exposure though viral RNA was detected in some tissues at day 27.

CODA in Belgium has several ongoing/completed studies addressing FMDV transmission. They have confirmed that infected Asian buffalo are able to transmit the virus to both naïve buffalo and naïve cattle, but that this transmission could be minimized by appropriate vaccination. Also considering wildlife, while published data show the capacity for transmission from wild boar, it seems that their actual contribution (as for gazelle) is highly variable in outbreak situations. Similarly, it appears that the contribution of environmental contamination of the virus is considerable in some situations, highlighting the need for development of effective disinfectant protocols in the field.

The OVI, in South Africa, is involved in a consortium (funded by Oregon state university and the Pirbright institute) research project to understand how FMDV is maintained and transmitted within the African buffalo. Central to the project is the detailed study of a captive herd of buffalo over 3 years, which will include monitoring of FMDV population dynamics, antigenic variation, transmission patterns, and the interaction of these factors with secondary influences (co-infection with common respiratory pathogens and/or seasonal malnutrition).

Researchers from the Pirbright Institute plan to conduct controlled transmission studies to provide accurate data that will inform next-generation modelling. By infecting cattle they will define the relative importance of different sources of virus emission, identify dominant routes of virus transfer between infected and naïve animals, and establish how the virus is most frequently taken up prior to infection.

PIADC, in collaboration with The National Veterinary Research and Quarantine Service, Korea, is studying the pathogenicity of Korean FMDV isolates, A/ROK/2010 and O/ROK/2010 in pigs and cattle with the specific aims of defining the time course of the disease and transmission windows, and to produce a set of reference data and materials for further characterisation. Working with the National Agricultural Research Center (NARC) of Pakistan, studies are also underway to define the role of persistently infected cattle and riverine buffalo in the persistence and transmission of FMDV.

Groups working in the Netherlands at the Wageningen University have highlighted the potential importance of environmental contamination during FMDV transmission between cattle, finding that if the virus persists environmentally for a day or more, it can account for around 30% of transmission, which had been previously under-estimated. Virus in the saliva of infected cattle can not only transmit to other cattle, but studies also showed that other small ruminants may be similarly susceptible, though precisely how the virus is transmitted is a topic of further investigation. The broader scope of work also includes improved understanding of FMDV transmission between vaccinated and non-vaccinated/recently vaccinated sheep, cattle and buffalo as well as quantification of viral load in different excretions and secretions from these animals. The aim is to develop a comprehensive understanding of the transmission pathways between animals of the same or different species under conditions of differing immunity and/or in the field. In parallel, the data generated will be incorporated into improved transmission models.

For a summary of research updates from contributing institutions, see appended Table 2.

### **Research priorities**

Our understanding of FMDV persistence has been revolutionised by finding that retention of virus in the lymph node appears to be more the exception than the rule, even in species not thought capable of retaining live virus, such as swine. What remains to be seen is whether this phenomenon is primarily to the advantage of host or pathogen.

Understanding early events during infection has been hampered historically by widespread use of non-physiological routes of challenge. Data now show that widespread adoption of more relevant routes of infection for laboratory studies is both feasible and desirable. Standardisation and universal acceptance of this approach would be beneficial.

Beyond expression of integrins, evidence of other determinants of susceptibility at the tissue and cellular levels have gained interest in recent years and should be pursued.

Factors underpinning genetic resistance and age-related susceptibility to FMDV infection warrant investigation.

The factors determining the extent of the role of wildlife species in field conditions during outbreaks should be defined with the support of laboratory controlled infections to enable full understanding of the disease in non-agricultural species.

It will be interesting to see to what extent the molecular virulence determinants identified *in vitro* are applicable in target species, and in particular, whether the same determinants are equally important for different species.

## Immunology

### From the 2010 Gap Analysis

Priority research aims stated were to:

1. Study mucosal responses to acute and persistent infections in cattle
2. Establish the immune mechanisms underlying protection to FMDV during the time-course of infection
3. Study neonatal immune responses to infection and vaccination and the influence of maternal immunity in protection and vaccine efficacy
4. Support research on the immunological mechanisms of cross protection in susceptible species
5. Determine the role of cellular innate immune responses in FMDV infection of cattle and swine
6. Develop methods to activate cells of the innate response to anti-viral activity (NK cells, T cells, and DCs)



7. Contract the development of antibodies to surface markers of critical immune bovine and porcine cell types as well as specific for bovine IFN $\alpha$  and  $\beta$  as well as porcine IFN $\alpha$
8. Contract the development of antibodies to surface markers of critical immune bovine and porcine cell types
9. Support basic research to understand the Type I interferon locus in cattle and swine and how the protein products of these genes affect innate and adaptive immune responses
10. Determine the differential expression of the IFN $\alpha$  genes in bovine and porcine
11. Develop technologies for analysing the adaptive immune response to infection and vaccination
12. Determine correlates between cellular immune responses and vaccine efficacy

## Literature review

### *Role of B cells*

Illustrating the importance of basic knowledge in FMDV target species, only recently have we discovered that the bovine immune system generates antibody diversity in a fundamentally different way than other species (Wang, Ekiert et al. 2013), thus bovine antibodies may be able to bind epitopes in a way that cannot be modelled using conventional parameters or in rodents. This knowledge could have profound implications for the way we consider designing vaccines targeting antibody responses, and might be one reason that vaccines can under-perform in cattle when developed in mice.

→ Functional implications of new knowledge on bovine antibody diversity should be elucidated *in vivo*. As part of the broader issue of small animal models, caution should be used when considering the applicability of antibody responses generated in rodents to likely outcomes in cattle.

Several studies have also advanced knowledge of the FMDV-specific antibody response. Recently, Pega et al described the kinetics of the early B cell response to aerosol infection, showing that FMDV-specific antibody secreting cells were present in the lymphoid tissue of the respiratory tract and spleen from 4 days post-infection (Pega, Bucafusco et al. 2013). The authors provide evidence to show that IgM is abundant early, both locally and systemically, and is responsible for viral clearance independent of T cell help. A study using porcine cells has also highlighted the potential importance of non-neutralising opsonic antibodies that facilitate uptake of bound FMDV by dendritic cells (DC), which are potent immune modulators. Summerfield's group provide evidence that opsonizing antibodies can increase plasmacytoid DC-mediated release of antiviral Type I IFNs in response to both homologous and heterologous serotypes (Lannes, Python et al. 2012). An earlier study in cattle also showed

significant increases in FMDV-specific recall responses *in vitro* using DC incubated with immune-complexed UV-inactivated virus (Robinson, Windsor et al. 2011).

→ Virus-neutralizing titre can no longer be considered the sole readout of the value of the antibody response to FMDV; there is sufficient data to warrant a thorough investigation of the role of immune complexes *in vivo* in target species during infection and vaccination, and how they might be exploited to improve immunity.

### *Role of T cells*

The dominance of T cell-independent antibody responses during early FMDV infection is well established, though the question remains: are T cell-dependent antibody responses simply not stimulated to the same extent, or are they actively inhibited? In mice, there is evidence that early Type I IFN production is responsible for diminished proliferation of T cells during infection (Langellotti, Quattrocchi et al. 2012). In the same model, chemically-inactivated virus also induced a regulatory T cell response. Recent data in cattle have confirmed the likely importance of this observation: Guzman et al provided the first conclusive data that bovine gamma-delta T cells are the regulatory equivalent of murine and human Foxp3-positive T regs, and also demonstrated the ability of these cells to significantly impede FMDV specific CD4 and CD8 T cell proliferation *in vitro* (Guzman, Hope et al. 2014). These findings may explain earlier *in vivo* observations, where partial depletion of gamma-delta T cells shortened the period of viraemia during FMDV infection of cattle (Juleff, Windsor et al. 2009). T regulatory cells have now also been defined and characterised in swine (Kaser, Gerner et al. 2011), though their functional significance for FMDV infection and vaccination has yet to be investigated.

→ The potential importance of regulatory gamma-delta T cells in the response to vaccination of cattle has been underestimated until now, and remains unknown in the case of other target species. Their impact on the magnitude of the T cell response to FMDV infection and vaccination should be investigated, with a view to minimizing their activation through rational vaccine design.

A second perspective on the relative weakness of the anti-FMDV T cell response comes from studies on their interactions with dendritic cells (DC), which license T cell activation and proliferation. FMDV appears to deplete splenic DC in mice (Langellotti, Quattrocchi et al. 2012), while bovine DC are effectively killed by live FMDV immune complexes *in vitro* (Robinson, Windsor et al. 2011). This could represent an immune evasion strategy, as the immune complexes formed by FMDV and host antibodies early in the response have the potential to destroy any DC attempting to take up the complexes and use the digested viral proteins to stimulate FMDV-specific T cells. Such a mechanism is consistent with *in vivo* observations in cattle, where there is little or no induction of the FMDV-

specific T cell response, while responses to other antigens are unaffected by infection (Windsor, Carr et al. 2011).

It also seems that different mechanisms of T cell suppression may predominate in different species. High levels of interferon alpha were linked with early T cell inhibition during FMD in mice (Langellotti, Quattrocchi et al. 2012), and a similar mechanism may operate in swine, at least during classical swine fever virus infection (Summerfield, Alves et al. 2006). In contrast, in cattle, only low levels of Type I IFNs are detected (Windsor, Carr et al. 2011), again consistent with the lack of immunosuppression that is evident in other species.

At least for vaccination, the importance of CD4-expressing T cells is now clear: depleting CD4 T cells from cattle significantly reduced virus-neutralising antibody titres and delayed antibody class switching (Carr, Lefevre et al. 2013).

→ These data emphasize the importance of T cell responses to optimize vaccine-induced immunity, which has been overshadowed by the traditional focus on neutralizing antibodies alone.

#### *Role of CD8 T cells*

Successful responses against many viruses rely on CD8 T cells, but their role in FMDV immunity remains obscure. Infecting swine with FMDV A24 has now been shown to induce specific CD8 responses, and moreover, a vaccine able to induce FMDV-specific CD8 T cells, critically, in the absence of marked antibody responses to the virus, diminished clinical signs and lowered viraemia (Patch, Kenney et al. 2013). Several CD8 T cell epitopes have also been identified within the structural proteins of FMDV A24 (Pedersen, Harndahl et al. 2013), though whether the responses detected in swine are in fact targeted towards these epitopes remains to be seen. While the role of CD8 T cells in cattle appears to be minor, the novel approach adopted by Plum Island researchers has yet to be tested in other species and may prove uniquely well-suited to answering the question in a way that other studies have been unable to.

→ FMDV specific CD8 T cells may be able to reduce the severity and duration of disease, making their stimulation a potentially useful adjunct to traditional vaccine strategies. Their role in species other than swine may warrant re-examination using new approaches.

#### *Neonatal immunity to FMDV vaccines*

The neonatal immune system is to some extent immature or perhaps even actively tolerogenic, as a result of *in utero* programming, and this can prove problematic for effective vaccination of young

animals. Moreover, the influence of maternally-derived antibodies delivered through colostrum upon subsequent immunization is far from clear. Effective protection of this vulnerable population group will require thorough knowledge of both these aspects of early life immunity.

Comparing groups of vaccinated calves with either high or low levels of anti-FMDV antibodies from colostrum revealed that abundant maternal antibody may prevent the development of the anti-FMDV IgM response, and could interfere with the production of virus-neutralising antibodies. These effects could not be overcome by a booster dose of the vaccine; however, the presence of maternal antibodies showed some benefit in terms of longevity of the overall anti-FMDV response measured by liquid-phase blocking ELISA (Bucafusco, Di Giacomo et al. 2014). The authors highlighted the somewhat contradictory findings when considering the data from blocking ELISA versus measurements of virus-neutralising titre, and proposed that VNT data might be the more useful in a young bovine population. They did not address the neonatal T cell response to vaccination.

In agreement with these findings, two week old calves without maternal antibodies to FMDV exhibited poor humoral responses to the same vaccine that was given to their mothers during pregnancy (Dekker, Eble et al. 2014). Interestingly, using an intra-typic heterologous vaccine, calves carrying maternal antibodies were able to mount a limited response, leading the authors to propose this as a strategy in emergency vaccination protocols in regions that were already employing prophylactic vaccines.

One study did detect induction of high levels of virus-neutralising antibodies in calves with maternal antibodies against FMDV, but only using oil- and not aluminium hydroxide- based adjuvants (Patil, Sajjanar et al. 2014). While the overall findings are perhaps inconsistent with those of other groups, this work does highlight the unaddressed issue of potential differences in efficacy of various adjuvants in young animals.

From outside the field, an intriguing study found that a Human Adenovirus 5-vectored swine influenza vaccine was able to overcome interference by maternal antibodies (Wesley and Lager 2006). It will therefore be particularly interesting to discover whether the same phenomenon occurs in calves vaccinated with the newly-licensed adenoviral FMDV vaccine (see Vaccines).

→ The neonatal immune system of FMDV target species remains far less well characterised than their adult counterparts. Neonatal calves seem able to mount responses to commercially-available FMDV vaccines, but only in the absence of high levels of maternal antibody, and perhaps only with the “right” adjuvant. Further study of neonatal immunity, with particular reference to the T cell compartment, in conjunction with side-by-side comparisons of responses to different adjuvants is required.

### *Correlates of protection following vaccination*

In line with the emerging importance of the T cell response to both FMDV infection and vaccination, new approaches to predicting protection following immunization are beginning to incorporate markers of T cell immunity. For example, combining VNT data with measurements of the levels of IFN $\gamma$  produced by CD4-expressing T cells in whole blood restimulation assays, allowed correlations to be seen that were associated with clinical protection following vaccination (Oh, Fleming et al. 2012). Interestingly, the extent of the IFN $\gamma$  response to the two vaccines tested was not dose dependent, and did not always reflect the VNT of the animal.

→ This raises the question of which other host-intrinsic factors are involved in determining the magnitude of the IFN $\gamma$ -producing T cell response to vaccination, and how the response might be augmented by rational improvements to vaccines.

Predicting cross-protection between vaccine strains and field isolates is a second important objective. Traditionally the likelihood of cross-protection has been estimated by virus neutralization test (VNT), liquid phase blocking ELISA and complement fixation assay, but these techniques are labour intensive and can be sub-optimally accurate. A recent study assessed the suitability of high-throughput avidity and IgG subtype ELISAs to predict cross protection in cattle from serum samples. In cattle immunized against FMDV A24 Cruzeiro and challenged with A/Arg/01, protection against the heterologous strain was associated with higher avidity antibodies to A/Arg/01. Some animals had low or undetectable VNT, yet were protected upon challenge, and this phenomenon was linked to a higher IgG1/IgG2 ratio than non-protected animals (Lavoria, Di-Giacomo et al. 2012).

→ Combining VNT and measurements of antibody avidity with immunoglobulin class ratios may well improve the accuracy of current vaccine-matching approaches. Moreover, understanding how to achieve optimal IgG ratios and increase immunoglobulin avidity could improve vaccine design, particularly where cross-protection is a priority.

The challenges of identifying correlates of protection following vaccination often reflect the more fundamental lack of knowledge of the events immediately following the administration of the vaccine, long before the emergence of measurable adaptive immune parameters such as neutralising titre. In the case of adenovirus-based FMDV vaccines, some progress has been made by the development of recombinant adenovirus 5 (rAd5) carrying either a luciferase- or GFP- encoding gene in place of FMDV sequences. Inoculation of the traceable rAd5-luciferase particles into cattle revealed the association of transgene products with antigen-presenting cells within the inflammatory infiltrate, and from 6 hours post-inoculation, within the inter-follicular areas of the draining lymph node (Montiel, Smoliga

et al. 2013). Experiments using rAD5-GFP revealed that the addition of an oil-based adjuvant increased the frequency of expression of the fluorescent protein within lymph migratory DC; in a separate experiment, the addition of the same adjuvant resulted in increased frequencies of FMDV-specific IFN $\gamma$ - and TNF $\alpha$ - secreting memory T cells compared to vaccination with rAD5-FMDV alone (Cubillos-Zapata, Guzman et al. 2011). Taken together these studies illustrate the potential of recent advances in our ability to dissect the interaction of vaccine components with antigen-presenting cells and the impact such interactions can have on ensuing immunity.

→ Such innovative reagents will prove useful in identifying the key immune cells involved early in the induction of the immune response to vectored vaccines, and provide a rational basis on which to compare the efficacy of different strategies during this critical initial phase of the vaccine response.

### Research updates

Researchers at DTU in Denmark have undertaken a package of work to both identify the FMDV structural protein peptides that can be bound by class I swine leukocyte antigen alleles, in collaboration with PIADC, and to define the epitopes capable of enhancing the anti-structural protein response.

INTA are collaborating with PIADC to understand the development and the protective role of the memory B-cell response in lymphoid tissues and nodes of the bovine respiratory tract following parenteral administration of FMD vaccine. While the vaccine is known to induce both systemic and mucosal immune responses, the underlying mechanisms are unknown, and as improved local respiratory tract immunity holds potential to increase protection, this understanding could prove valuable in informing improved vaccine/adjuvant design in the future. In parallel, INTA and PIADC are also developing techniques to improve our ability to study the induction of adaptive immunity in the mucosa following aerosol infection. They aim to establish the time course of parallel induction of mucosal and systemic immunity, and to understand the importance of distinct antibody isotypes for local *in vivo* neutralisation of FMDV in cattle. Alongside, technical expertise transferred from the Pirbright Institute has enabled INTA scientists to embark on a comparison of the molecular responses induced in murine DC and bovine afferent lymph DC by exposure to either live or inactivated FMDV. This will contribute substantially to our appreciation of the very earliest steps of initiation of immunity to FMDV by vaccination or infection, and the comparative nature may shed light on the extent to which we can hope to accurately model bovine immunity at the cellular level in the murine setting.

INTA scientists are also investigating non-classical correlates of protection against heterologous challenge. In combination with traditional VNT/ELISA, researchers plan to characterise the specific serological responses of cattle to vaccination in terms of antibody avidity and isotype profiles, as well as cellular immune parameters. This will then be compared to the results of heterologous challenge in an attempt to identify the factors most relevant for protection.

Directly addressing the need for improved knowledge of the factors impacting neonatal responses to FMD vaccines, INTA are also planning to define the immune responses of calves in both the presence and absence of colostral immunity. As well as valuable information on the composition and kinetics of colostral-derived immunity in calves, this study will, for the first time, interrogate the effects on cellular immunity in calves and should generate much needed data in this under-studied area.

PIADC are leading several research projects that will substantially enhance our understanding of FMDV immunity: with INTA, they plan to focus on identifying the genetic basis of response levels of FMDV infection and vaccination and to what extent these responses are heritable, and in a separate collaboration, to exploit *in vitro* models of FMDV-specific antibody production to determine the companion cell types likely to be important *in vivo*.

For a summary of research updates from contributing institutions, see appended Table 2.

### Research priorities

New knowledge on the bovine immunoglobulin response requires interpretation for its relevance to FMDV infection and vaccination in natural FMDV target species.

The importance of non-neutralizing antibodies in the response to FMDV has become apparent, particularly in terms of interactions of antibody-bound virus with immune cells. We now need to define the roles of these immune complexes *in vivo* in target species to enable their exploitation.

Regulatory gamma-delta T cells now appear to play a significant role in the response to vaccination of cattle. Similar experiments should be undertaken in other target species to understand the impact of this cell type to FMD vaccination in broader terms, particularly in light of new data highlighting the importance of T cell responses to optimize vaccine-induced immunity. Similarly, CD8 T cell stimulation may also be a desirable outcome of vaccination, but its potential remains unexplored outside of swine.

The neonatal immune systems of FMDV target species remain far less well characterised than their adult counterparts. Further study of neonatal immunity, with particular reference to the T cell

compartment, in conjunction with characterisation of FMDV vaccine and adjuvant responses is required.

Qualitative hallmarks of effective adaptive immunity to FMD vaccines and infection are being uncovered. Understanding how to use this knowledge in a more comprehensive approach to vaccine matching may prove invaluable. Alongside, further study to determine how to achieve optimal IgG class ratios and increase immunoglobulin avidity should inform improved vaccine design, particularly where cross-protection is a priority.

Mucosal immune responses remain relatively under-studied in target species. Robust techniques should be developed and applied to understand how mucosal and systemic immunity combine to provide effective protection following vaccination or during infection.

## **Molecular Biology**

### **Literature review**

Defining the molecular features of FMDV has direct applications for how we understand, and therefore overcome, infection with the virus, as well as identifying molecular targets that may be amenable to therapeutic inhibition in order to prevent viral replication, characterising the effects of the virus upon host cells allows us to appreciate its immune evasive strategies.

#### *Cell entry*

At the stage of cell entry, the main new finding is the requirement for phosphatidylinositol 4,5 bisphosphate (PIP<sub>2</sub>) within the plasma membrane for integrin-dependent FMDV entry (Vazquez-Calvo, Sobrino et al. 2012). This is particularly interesting as PIP<sub>2</sub> has since been linked to regulation of the autophagosome/lysosome system (Rong, Liu et al. 2012), which has emerged as an important component of FMDV infection on the cellular level. FMDV induces the formation of autophagosomes to facilitate cell entry (Berryman, Brooks et al. 2012), and FMDV 2C protein has been shown to interact with Beclin1, a host protein that regulates the autophagy pathway, perhaps as a means of preventing lysosomal fusion (Gladue, O'Donnell et al. 2012).



As well as the importance of autophagosomes for cell entry, FMDV also requires cellular membranes for genome replication. It now seems that these membranes originate from the early secretory pathway, specifically the pre-Golgi compartment (Midgley, Moffat et al. 2013), which may link with the observation that FMDV 3C(pro) can cause fragmentation of the Golgi associated with a loss of microtubule organization (Zhou, Mogensen et al. 2013). These disruptions to vesicular trafficking and the secretory pathway are particularly relevant when it comes to the production and release of cytokines from infected cells, and the transport of immune-stimulatory MHC/antigen complexes to the cell surface. Rearrangements to the internal membrane system of infected cells thus appear to serve the dual purposes of facilitating replication and avoiding immune stimulation.

#### *Immune evasion*

In addition, FMDV has evolved several specific mechanisms to evade the host type I IFN response. While earlier work identified roles for FMDV L(pro) and 3C(pro) (Wang, Fang et al. 2012) in inhibiting the production of type I IFN, new data show that 3C(pro) can also impede signalling downstream of the type I IFN receptor, specifically the nuclear translocation of STAT1 and STAT2 (Du, Bi et al. 2014). These molecules have hundreds of potential transcriptional targets, including many anti-viral effectors and immune-stimulatory genes. Alongside, studies in tumour cell lines have identified potentially relevant alterations to cell signalling induced by FMDV VP1 binding to target cells, including down-regulation of COX-2/PGE2 and IKK/NF- $\kappa$ B signalling, both of which are important immune pathways (Ho, Hung et al. 2014).

→ Taken together these studies highlight the varied and powerful ways in which FMDV manipulates the host immune system to permit its replication and spread, and validate the development of biotherapeutics to counteract such effects during the critical early days of infection risk in the outbreak scenario.

#### *Molecular virulence determinants*

Alongside host determinants of the virulence of FMDV infections (see (Arzt, Pacheco et al. 2014), under Pathogenesis), molecular features of the virus can have profound impacts on the virulence of the strain. Attenuation has been linked with both low- and high- fidelity variants of the viral RNA-dependent RNA polymerase, the latter of which may act by limiting the potential for adaptive quasi-species emergence (Xie, Wang et al. 2014, Zeng, Wang et al. 2014). In addition, an elegant series of *in vitro* experiments tracked the differential virulence of two A serotype field strains isolated during the Argentine outbreaks of 2000/1 to differences in the structure of the internal ribosome entry site (IRES) of the viral genome (Garcia-Nunez, Gismondi et al. 2014). Interestingly, the interaction between the

IRES and viral 3' untranslated region (UTR) was the key determinant of the level of FMDV replication, adding a second level of regulation to the system.

The roles of FMDV non-structural protein 3A in virulence now appear to be multiple and varied: optimal FMDV 3A dimerization has been linked to viral infectivity, at least *in vitro* (Gonzalez-Magaldi, Postigo et al. 2012), while the interaction of 3A with the host protein DCTN3, likely involved in intracellular organelle transport, seems to be a potent virulence determinant in both primary bovine cell cultures and in cattle (Gladue, O'Donnell et al. 2014). Moreover, while deleting amino acids 87-106 of the FMDV non-structural protein 3A did not affect virulence in swine, in cattle these mutations resulted in limited replication in the pharyngeal area after aerosol infection but no systemic spread (Pacheco, Gladue et al. 2013).

→ Thus FMDV 3A warrants further investigation as a determinant of both virulence and host tropism. The functional effects of the described mutations should be characterised in order to understand the basis of their effects on *in vivo* virulence.

#### *Molecular effects of infection*

Our knowledge of the impacts of FMDV infection upon target cells has been advanced by two notable studies. As well as direct repression of gene transcription, FMDV infection can alter host cell gene expression via modulation of cellular micro-RNAs (miRNAs). In the PK-15 porcine kidney cell line, FMDV Asia 1 induced differential expression of 172 known, and 72 novel, miRNAs after 6 hours, compared to non-infected cells. Pathway annotation revealed that the miRNAs were abundant in cell death and immune response pathways (Zhang, Liu et al. 2014). In addition, another group working in China employed high-throughput quantitative proteomics to analyse the global changes in protein expression in FMDV-infected IBRS-2 cells. After 6 hours of infection, 77 cellular proteins were significantly up-regulated, and 50 were significantly down-regulated, relative to non-infected cells. Ingenuity pathway analysis highlighted the abundance of regulated proteins involved in various biological pathways including cell movement, intercellular signalling, lipid metabolism and protein synthesis (Ye, Yan et al. 2013).

→ These studies represent an exciting new era in the application of precise, quantitative, high-throughput molecular techniques to the study of FMDV. This unbiased approach holds great promise in terms of understanding the molecular pathogenesis of FMDV infection and identifying new therapeutic targets for intervention. Future study should focus on the development of improved controls for infection (or the use of purified virus), and movement away from cell lines. Early time points of infection would be revealing, as would the use of primary immune cells as targets. The extent

to which changes observed are driven by the cellular response to virus versus actively induced by replication could be addressed by the use of purified inactivated virus, while questions regarding cross-species differences in susceptibility could also be addressed at this level.

#### *Understanding capsid stability*

As seen in the Vaccines section, major advances have been made using FMDV capsids engineered to have increased resistance to low pH. This has required some understanding of the molecular requirements of acid-resistance: two separate studies on mutants of Type Asia1 or Type C FMDV with increased resistance to low pH allowed the identification of parallel single amino acid substitutions at VP1 N17D and VP2 H145Y (the residue required for VP0 cleavage) that were key for the acid-resistant phenotype (Vazquez-Calvo, Caridi et al. 2014, Wang, Song et al. 2014). Type O FMDV required only the VP1 N17D substitution to achieve acid-resistance, which substantially increased its immunogenicity under acidic conditions and highlighted the potential of this alteration to improve vaccine production (Liang, Yang et al. 2014).

#### **Research updates**

Following the collaborative project between PIADC (USA), the CBMSO (Spain) and INTA (Argentina) that identified the structure of the internal ribosome entry site (IRES) of the viral genome as a molecular virulence determinant (Garcia-Nunez, Gismondi et al. 2014), preliminary work has also uncovered evidence of a potential virulence determinant from two strains of the virulent subtype that is evident in murine infection but not reflected in differences in replication rate in cell culture. Sequence comparison has now identified 6 amino acid changes across viral proteins VP2, VP1 and 2C as well as 26 synonymous changes throughout the viral genome that are being evaluated for their impact on lethality of FMDV in adult mice.

DTU are conducting a program of research aimed at improving molecular knowledge of the virus, in particular they are aiming to understand: the requirements for capsid precursor processing and capsid assembly to assist vaccine development, and how to produce self-tagged virus particles through modification of a 3C protease cleavage site.

In a collaborative effort between PIADC and IZSLER, FMDV replication is being analysed from the perspective of the host cell. Aiming to identify the host proteins that play critical roles in the process of virus replication and to characterize their cellular location, interaction kinetics, and role in the viral life cycle, this project may offer new insights and possible points for intervention during infection.

Further refinements to our knowledge of FMDV's interaction with the type I IFN system are being sought through research at the FLI, where *in vitro* assays aim to identify viral and host protein interaction partners and to understand their significance within the host cell signalling pathways.

For a summary of research updates from contributing institutions, see appended Table 2.

### **Research priorities**

The multiple and varied ways that FMDV manipulates the host immune system are becoming evident from molecular studies which should serve to both validate and direct design of novel biotherapeutics.

Substantial data indicate that FMDV 3A warrants further investigation as a determinant of both virulence and host tropism, but an emphasis on experimentation in natural host species is required moving forwards.

Exploitation of precise, quantitative, high-throughput molecular techniques to study FMDV offer great promise for advances in understanding of pathogenesis at the cellular level. Their future application to primary cells or more physiologically-relevant cell lines, combined with careful experimental design, will be required for this promise to be realised.

Considering recent advances in our molecular understanding of FMDV's interactions with the host IFN response, we now need to establish to what extent we can link molecular findings with whole organism responses to natural infection. As a starting point, some experiments carried out in immortalised cell lines should be repeated in cell lines and/or primary cells from relevant target tissues of natural host species.

### **New Research Tools and Approaches**

#### **Literature review**

Cell lines are appropriate for preliminary investigation of the molecular events of infection, and in particular may be effective screens for potential biotherapeutics aimed at rendering target cells resistant to FMDV. There is some evidence that porcine tonsil cells may be applicable for the evaluation of oral, low-dose IFN- $\alpha$  treatments (Razzuoli, Villa et al. 2014), and would seem a physiologically relevant choice for testing other agents administered either orally or by aerosol.

Facilitating improved study of FMDV, new antibodies recognising the NS protein 3AB (Lin, Shao et al. 2012), and VP1 of type A and type O VP1 (Cho, Jo et al. 2012) have recently been characterised.

### Research updates

INTA are leading developments in reducing the need for large animal challenge during vaccine potency testing through refining trials in mice. The current project aims to measure the correlation between vaccine-induced antibody titres in cattle and mice, and the extent to which isotype ratios and avidity indices are similar in the two species.

Our understanding of the immune response to both infection and vaccination against FMDV is hampered by an incomplete knowledge of the immune system of natural target species, which is in turn compounded by a limited number of reagents with which to study them. PIADC are improving reagent availability both directly through the development of new monoclonal antibodies for bovine cytokines and immune cell markers, and indirectly, by importing, testing, and making-available monoclonal antibodies for bovine immune cell surface proteins previously produced at the International Livestock Research Institute (ILRI) in Kenya. Reagents available for immunological study of veterinary species can be identified at <http://www.immunologicaltoolbox.co.uk> and <http://www.umass.edu/vetimm/>

For a summary of research updates from contributing institutions, see appended Table 2.

### Research priorities

Large animal experimentation for FMDV is necessary, but expensive, and suffers from multiple practical limitations. Appropriate *in vitro* and small animal models of the disease have the potential to advance knowledge and reduce the number of large animals needed, but the challenge of developing and validating the “right” model remains. In parallel, reagent/method development to enable in-depth study of the immune systems of target species, particularly the mucosal compartment, would facilitate advances in FMDV pathogenesis and vaccine science.

## Conclusions

FMD control is constrained by the tools available. Progress accelerates with scientific breakthroughs and stagnates in the face of unsolved problems. Progress in disease control can take several forms:

- Achieving control where previously not possible or allowing a better level of control.
- Achieving the same level of control but in a more efficient way (time, cost).
- Allowing increased certainty in disease/infection status, which facilitates control and allows access to FMD free markets, thereby increasing incentives to control FMD.
- Providing increased understanding of a disease, which can improve our ability to describe and predict events, or may eventually result in tools for control not anticipated at the time of discovery.

Historically our ability to control FMD has depended on the ability to coordinate control through effective veterinary services with control facilitated by the development of imperfect but sufficiently effective vaccines, which have changed relatively little in the last half-century. More recently our ability to select an effective control strategy has been enhanced by the development of mathematical models. Improved diagnostics enable us to detect infected animals in vaccinated populations with greater confidence, allowing for less draconian standards for international trade and reduced dependency on culling based strategies. Further refinements are required in these areas.

Currently available tools for FMD control have enabled eradication in much of the developed world, however, in many developing countries FMD remains uncontrolled, and with three-quarters of world's livestock living in endemic regions this represents a vast unmet need. In these settings the biosecurity measures that have been fundamental to successful FMD control in the developed world can seldom be implemented effectively. Improved vaccines, with longer-lasting protection against a wider range of strains and lower production costs could therefore be the single most important development to allow FMD control where previously not possible. Although this is not imminent, encouraging progress has been made with several novel vaccine candidates addressing key weaknesses of the current inactivated vaccines. While new discoveries are crucial, uptake and implementation of existing methods and technologies is often lacking, and the same compliance frameworks will be crucial for the success of any novel strategies that emerge. Lessons learnt about how to control FMD with existing quality assured vaccines should be shared and clearer guidance provided to those struggling to control the disease despite significant efforts.

Another area of huge potential is genetic and molecular research. More powerful tools and analyses are promising to increase our understanding of various aspects of FMDV evolution, ecology and epidemiology. This in turn will benefit a range of areas, from basic virology, to vaccine and diagnostic development. Furthermore improved genetic technologies have the potential to reveal information crucial for control, such as transmission chains, vaccine match, and level of virus circulation.

FMD control has been prioritised by many governments around the world. Increasingly, research is being conducted outside the traditional bastions of the established FMD institutes in Europe, North and South America, with notable work being done in China and India, and increasing research capacity within Africa. Experiences in South America and Europe have shown that through decades of sustained investment in FMD research and control, eradication is achievable - even in regions where FMD is rampant and control seemingly impossible. Although global control seems far off, progress is being made and there is cause for optimism.

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## Appendices

### Contributing Institutions & Financial Support

Abbreviation	Institution	Country
AHL-NZ	Animal Health Laboratory	New Zealand
ANSES	Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail (National Agency for Food, Environmental and Occupational Health Safety)	France
BVI	Botswana Vaccine Institute	Botswana
CEVAN	Centro de Virología Animal (Center of Animal Virology)	Argentina
CIRAD	Centre de Coopération Internationale en Recherche Agronomique pour le Développement (French Agricultural Research Centre for International Development)	France - International
CODA	Centrum voor Onderzoek in Diergeneeskunde en Agrochemie (Veterinary and Agrochemical Research Center)	Belgium
CSIRO	Commonwealth Scientific and Industrial Research Organisation	Australia
DTU	Danmarks Tekniske Universitet (Technical University of Denmark)	Denmark
EUFMD	The European Commission for the control of Foot-and-Mouth disease	Italy
FLI	Friedrich-Loeffler-Institute	Germany
INTA	Instituto Nacional de Tecnología Agropecuaria (National Institute of Agricultural Technology)	Argentina
IVI	Institute of Virology and Immunology	Switzerland
IVRI	Indian Veterinary Research Institute	India
IZSLER	Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna (Lombardy and Emilia Romagna Experimental Zootechnic Institute)	Italy
LANAVET	Laboratoire National Veterinaire (National Veterinary Laboratory)	Cameroon
LVRI	Lanzhou Veterinary Research Institute of the Chinese Academy of Agricultural Sciences (CAAS)	China
NADDEC	National Animal Disease Diagnostics and Epidemiology Centre	Uganda
NaLIRRI	National Livestock Resources Research Institute	Uganda
NARC	National Agricultural Research Center	Pakistan
NCAD	National Centers for Animal Disease	Canada
NIAB	Nuclear Institute for Agriculture and Biology	Pakistan
NIBGE	National Institute for Biotechnology and Genetic Engineering	Pakistan
NVL	National Veterinary Laboratory	Pakistan
NVRI	National Veterinary Research Institute	Nigeria
	Onderspoort Veterinary faculty, University of Pretoria, South Africa	South Africa
OVI	Ondespoort Veterinary Institute	South Africa
ARS	Agricultural Research Service, USDA, PIADC	USA
APHIS	Animal and Plant Inspection Service, USDA, PIADC	USA
DHS	Department of Homeland Security, PIADC	USA
	Pirbright Institute	UK
	University of Glasgow	UK
	University of Minnesota	USA
Wageningen	Central Veterinary Institute (CVI) University of Wageningen	Netherlands
VLA	Veterinary Laboratory Agency	Tanzania
WCS-AHEAD	Wildlife Conservation Society - Animal & Human Health for the Environment And Development	USA – International
	Zoetis	International

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## Summary of Research Activities at GFRA and EuFMD Member Institutes

**Table 2:** Summary of reported recent, ongoing, or planned FMD research activities at responding GFRA and EuFMD institutes. Instead of listing as a separate category, activities relating the wildlife have been highlighted in red.

Institute	Diagnostics	Vaccine Quality Assessment	Epidemiology	Pathogenesis	Immunology	Vaccines	Antivirals	Molecular biology	Impact & trade	Other
AHL-NZ, New Zealand	-Evaluate diagnostic tests in red deer									
ANSES, France				-Cellular models of persistent infection				-Sequencing West African & Pakistan viruses		
BVI, Botswana								-Sequencing and phylogenetics of FMD in cattle & buffalo		
CEVAN, Argentina	-Serological tools for detection, population immunity and vaccine evaluation	-Alternative vaccine quality and potency tests -Typing and matching using serology, molecular and antigenic data		-Characterise protein-protein interactions during transcriptions and replication		<b>Vaccines</b> -Vector vaccines (replicative and non-replicative +/- cytokines) -Vaccines using viral replicons, bacterial phages with specific epitopes & identify novel immunogens		-Rapid detection and typing		
CIRAD, France			<b>Epidemiology</b> -FMD ecology & control at the wildlife-livestock interface in Southern Africa -SE Asia-surveillance evaluation & participatory surveillance							
CODA, Belgium	-Simplified ELISA tests -IgA & NSP ELISA validation -Luminex assay -Twinning project in Nigeria	- <i>In vitro</i> potency test -Vaccine NSP purity test -Measure of vaccine antigen payload	<b>-Wild boar &amp; gazelle</b> involvement in outbreaks -NSP surveillance -Modelling vaccine strategies -Evaluation of simulation models	-Transmission between sheep & cattle -Transmission from environmental contamination -Vaccination and transmission from Asian water buffalo to cattle	-Onset & duration of immunity -New DIVA vaccines	-Heterologous protection -Vector vaccines	-Antivirals proof of concept -Cost-effectiveness of antivirals	-Predicting heterologous protection		

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CSIRO, Australia	-Outbreak preparedness including diagnostic capabilities, strategies and reagents	-Potency testing of vaccine bank strains	-Identify strains with high risk of incursion and consequences -Develop vaccine control strategy			-Develop sequencing for tracing and vaccine selection				-Capacity building in endemic countries to reduce incursion risk
DTU, Denmark	-Develop serotype-specific RT-qPCR assays	-Role of leader protease in blocking host cell protein synthesis and infection	-Modelling outbreaks in free countries (Denmark)		-Identify epitopes that enhance immune response	<b>Vaccines</b> -Capsid processing & assembly -Analysis of endemic country strains & transmission between <b>species including wildlife</b> -Next generation sequencing & reverse genetics to assess diversity, adaptation & pathogenicity				
FLI, Germany	-Baited swab sampling in <b>wild boar</b> -Inactivation of FMDV by nucleic acid extraction buffers & optimised diagnostics -Non-invasive preclinical sampling (air sampling?)	-Heterologous potency tests for serotype A viruses with vaccine bank strains, data used for improved potency prediction		-Challenge study in <b>wild boar</b>	-How do FMD viral factors interfere with the IFN-I response?	-Viral vectors expressing different FMDV proteins as vaccine candidates			-FMD cannot survive in properly processed sausage casings	-Defining minimum bio-risk management standards for laboratories working with FMDV"

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INTA, Argentina	-New SAT2 ELISA to assess vaccine potency & coverage	-Mice for vaccine potency testing -Using old & novel assays, antibody avidity, isotype profiles & IFN $\gamma$ to predict heterologous protection -Measuring Ab vaccine response in buffaloes & pigs	-Population immunity studies & identification of immunity gaps	-Elucidating the viral determinants of differential pathogenicity of 2 A strains, considering molecular, structural characteristics and pathology	-Development and protective role of memory B-cells in lymphoid tissues and nodes+ mucosal Ab immunity of respiratory tract -Vaccine response in pigs assessed by LPB-ELISA, indirect and avidity ELISA, isotype ELISA and also cell-mediated immunity assessed by CSFE-LPA -Interaction between MAbs & different peptides from immune-dominant epitopes -Differences in intracellular mechanisms triggered by the infective particle or the inactive virus in murine dendritic cells (DC) and bovine afferent lymph DC -Colostrum immunity & vaccination	-VLPs using baculovirus-insect cell & mammalian cell transfection system -Novel adjuvants for pigs-matrix of soybean lecithin, cholesterol, glycans and saponins forming nanoparticles -Dendrimeric peptide-based FMD vaccines in cattle -Baculoviruses as adjuvants assessed in mice -Enhancement of responses to DNA-based vaccine by plasmids encoding CD40L and IL-15 assessed in mice	-RNAi-based antiviral tools	-Assess virus diversity, evolution & distribution during 2000/1 outbreak -Validity of phylogenetics based on one sample per outbreak.		
IVI, Switzerland						-Improving FMD vaccines for cross-protection, longer immunity and mucosal immunity				

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IVRI, India	-NSP ELISA with 3ABC protein expressed in insect cells using a baculovirus system -LFD test development -Relating results LPBE to VN				<b>Vaccine</b> -Baculovirus expression system for FMD vaccines -Reasonable guinea pig immune response to inactivated vaccine given with Toll-like receptor ligand, flagellin -Assess adenovirus vaccine comprising capsid coding sequences of Indian vaccine strains by serology and later challenge will be done		<b>Antivirals</b> -Adenovirus vector produced IFN $\alpha$ and $\lambda$ as therapeutics to control FMD at the early stage of infection -siRNA against FMDV 3C protease inhibited viral growth in BHK cells			
IZSLER, Italy	-Develop monoclonal antibodies for ELISA kits, LFD, Luminex	-ELISA for 146S measurement -SP antibodies to assess vaccine protection. -Harmonisation between laboratories	-NSP & SP sero-surveys for endemic countries (issues-cross-reactivity, user-friendly kits and matching of field and tests strains, correlation with protection)			-FMD structure & antigenic variability, epitope mapping				- Disinfection in lab and field environment
LANAVET, Cameroon			-Epidemiology of strains in Cameroon							
LVRI, China	-Improved diagnostics, including monoclonal ELISA & RT-LAMP (including for SAT viruses) - Immunochromatographic strip with NSP MAb and dot-blot kits developed				-Understanding mucosal immunity	<b>Vaccines</b> -Novel vaccines- nanoparticle-based nasal vaccine, epitope peptide vaccine, synthetic peptide vaccine, inactivated virus gene engineering based vaccine, and recombinant baculovirus capsid vector -Deleting an immunodominant epitope in NSP 3A for vaccine with complementary DIVA ELISA test -G-H loop epitope vaccine adjuvanted with and polyinosinic-cytidylic acid -VLP with <i>E.coli</i> expression system				
NADDEC, Uganda	-OIE twinning with Swedish Agricultural University									-Climate change resilience
NaLIRRI, Uganda			- Investigating FMD Risk factors							
NARC, Pakistan	-Develop RT-LAMP			-Understanding distribution of FMD in Pakistan -Role of persistently-infected water buffalo & cattle						

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<b>NCAD, Canada</b>	<b>Diagnostics</b> -Monoclonal antibodies for ELISA development-O typing -Monoclonal antibodies for serotype A cELISA & rapid tests -Polyspecific virus capture ApoH-ELISA for FMD & other vesicular diseases -Multiplex assay for detection of many swine viruses -FMD typing microassay			<b>Diagnostics</b> -Assays adapted for portable, automated instruments -Genetic engineering of cell lines for rapid replication of FMD virus for tests -Simplification of assays -Use of oral fluids for swine surveillance -146S ELISA to measure infectivity of culture						
<b>NIAB, Pakistan</b>	-Use and evaluation of PCR & RT-LAMP									
<b>NIBGE, Pakistan</b>	-IgM ELISA									
<b>NVL, Pakistan</b>	-RT-PCR assays for detection and serotyping of local FMDVs								-Molecular epidemiology of regional FMD	
<b>NVRI, Nigeria</b>	-Increasing diagnostic capacity		-Map FMD risk & strains & vaccine matching							
<b>Ondespoort University, South Africa</b>			- <b>African buffalo</b> behaviour & FMD risk							
<b>OVI, South Africa</b>		-Predict cross protection from capsid protein genetic & structural data +limitations of current matching methods -Identifying sites of antigenic drift for SAT1 viruses	-How FMDV is maintained in <b>buffalo populations</b> & the role of concomitant disease and viral drift -Distribution, typing & vaccine matching of FMD in Uganda			-Reverse genetics to produce a SAT vaccine seed virus with capsid stability and cell culture adaptation mutations				

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PIADC, USA	<p>-Infrared thermatography for early detection of FMD</p> <p>-Development of an inexpensive FMDV diagnostic kit based on camel monoclonal antibodies for sub-Saharan Africa</p> <p>-Multiplex RT-LAMP to diagnose FMD and Vesicular Stomatitis, with a LFD readout format.</p>	<p>-Determine challenge study protocols in sheep to assess vaccine protection and optimal route of vaccine application &amp; dose</p> <p>-Determine immune correlates and assess protection from the adenovirus vectored FMD vaccine in cattle</p>	<p>-Web-based FMD international surveillance platform (FMD Bioportal)</p> <p>-Modelling FMD transmission and evolution in the Lake Chad Basin</p> <p>-Transmission from carriers in the field</p> <p>-Phylogenetic, temporal and spatial analysis of viral strains circulating in Central Asia, Southeast Asia and Africa</p> <p>-Ecology &amp; epidemiology in Cameroon, vaccine selection and supporting control</p> <p>-Ecology &amp; epidemiology in Pakistan, vaccine selection and supporting control</p>	<p>- Understand host-pathogen interaction</p> <p>- Pathogenicity &amp; transmission of Korean2010 isolates in pigs and cattle</p> <p>-Pathology of FMDV from persistently infected cattle and Asian buffalo in Pakistan &amp; their role in transmission</p>	<p>-Define the immune response to infection &amp; vaccination and identify genetic characteristics associated with animals with differing response</p> <p>-Analyse T cell responses to FMDV infection in swine and cattle</p> <p>-Import cells producing monoclonal antibodies specific for characterised bovine cells from ILRI, Kenya, and test for purity from pathogen contamination</p> <p>-Breed-specific responses to FMD infection and vaccination for vaccine development</p> <p>-Characterise host proteins that play critical roles in virus replication</p> <p>-Define events and cell type requirements leading production of FMDV-specific bovine Ig's <i>in vitro</i></p>	<p>-Novel vaccines e.g. FMDVLL3B3D [see Zoetis]</p> <p>-Producing a recombinant vaccine seed virus with improved stability and cell-culture growth and adaptation.</p> <p>-Improving cDNA technology and identification of epitopes to produce better matched recombinant inactivated vaccines for Africa</p>	<p>-Unspecified antiviral research</p> <p>-Bio-therapeutics targeting NK cells with cytokine expressing adenovirus vectors</p>	<p>-Typing strains in Pakistan for use in vaccines</p> <p>-Typing strains in India for vaccine selection</p> <p>-Molecular epidemiology of FMDV in Vietnam</p> <p>-Molecular characterisation and distribution of FMD and training in Uganda for vaccine selection</p>	<p>-Identify &amp; evaluate chemical agents for viral inactivation</p>	

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<b>Pirbright Institute, UK</b>	- Strain specific PCR - Pen side PCR - RT-LAMP - Improved ELISAs	- Vaccine matching and strains for East Africa	- Vaccine effectiveness evaluation - Modelling within & between farm transmission - Modelling within host dynamics	- Viral receptors - Transmission mechanisms - Carriers & transmission & <b>African buffalo</b> - Picornavirus cell entry	- CD4 T cells & FMD immunity - Identifying functionally important FMD antigens	- Increased spectrum vaccines - Stable empty capsid vaccine		- Sequence evolution and transmission	- <b>Wildlife</b> , control & trade needs - Safe trade protocols - FMD economic impact	
<b>University of Glasgow, UK</b>	- Develop milk NSP test & PCR, use of virus sequence from milk to monitor FMD burden - Monitor vaccine field effectiveness - Diagnostics for East African strains & lab capacity in Africa		- Basic epidemiology of FMD in Tanzania including <b>wildlife</b>	<b>Immunology</b> - Testing the immunodominance of antigenic sites and the contribution of different constituent amino acid residues by reverse genetics & use for vaccine matching - Identification of protective antibodies to capsid epitopes that protect against all serotypes [b cell epitopes, conserved epitopes] - Correlates of immunity for vaccine batch testing including T cell response		- Improved vaccines for Africa & field evaluation - Novel adjuvants - Matching/vaccine strain selection based on capsid gene sequence		<b>Molecular</b> - Develop tools/skills to use next generation sequencing to infer epidemiology & virus evolution, for understanding & tracing - Understanding how within-host replication and transmission influence virus diversity and relationship with consensus sequencing and diversity along transmission chains		
<b>University of Minnesota, USA</b>			- FMD epidemiology in endemic countries - Modelling FMD in USA swine populations							

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University of Wageningen, Netherlands	-NSP Ab blocking ELISA -Timing of NSP antibodies after infection or vaccination in cattle & small ruminants	-Link between VNT & protection varies with dose & antigen -Vaccine selection/matching in Eritrea -Humoral & cellular immune correlation with vaccine protection -Harmonising tests used to predict efficacy - <i>In vitro</i> immunoassays to monitor NSP content and reduction during vaccine purification -Improved 146S quantification to assess vaccine batch quality	-Environmental virus accounts for 1/3 of transmission in housed cattle but nature of virus entry is unknown -Small ruminants are as susceptible as cattle but less infectious -Model implies 2-5 km ring vaccination can control an outbreak -Outbreak duration economically crucial so rapid control/vaccination needed -Serotypes in Africa/Eritrea -Assess FMD transmission in vaccinated <b>buffalo</b> - <b>Gazelle &amp; wild boar</b> role in FMD transmission -Validate FMD control models assessing population data, sensitivity to poor/missing data-different vaccination strategies	-Future work will assess relation between genome and blocking of IFN responses -Virus excretion in different species-cattle, sheep, pig, <b>buffalo, gazelle, wild boar</b>	-Timing of NSP antibodies after infection or vaccination in cattle & small ruminants	-How to improve antibody response by changes in vaccine composition, dose & route of administration				
VLA, Tanzania			-FMD epidemiology & farmer awareness							
WCS-AHEAD, USA-International			-Modelling risk of FMD product contamination along value chains -Modelling SAT serotypes <b>and African Buffalo</b>						-Socio-economic & environmental impact of FMD on development and <b>wildlife</b> , including options for commodity-based trade	
Zoetis, International	-Complementary NSP ELISA for a negative marker vaccine					<b>Vaccines</b> -Inactivated recombinant FMD virus vaccine platform with a negative marker, that is non-virulent with no transmission, with restriction sites to facilitate insertion of the capsid coding region of any serotype				



