# Global Foot-and-Mouth Disease Research Alliance (GFRA)


Global Foot-and-Mouth Disease
Research Alliance

# 2010 Report
# Research Activities Worldwide

**GFRA Website:** http://www.ars.usda.gov/gfra/

# Contents

# Background and Introduction to the Report

*The following report compiles the information provided by multiple contributors from FMD research laboratories distributed around the world. Consequently, the document should be used as providing a general guide to active research lines on FMD around the world but it should not be expected to be fully comprehensive or consistent in its level of detail.*

Foot-and-mouth disease (FMD) is a highly contagious and acute viral affliction of domestic and wild cloven-hoofed animals. It is a rather complex disease caused by a group of related but distinct viruses, collectively named FMD virus (FMDV) of the genus Aphthovirus in the family Picornaviridae. The seven distinct virus serotypes, i.e. A, O, C, Asia-1 and the Southern African Territories (SAT) types 1, 2 and 3, are distributed globally, though they have different geographic distributions and epidemiologies. The disease caused by these viruses is clinically indistinguishable and infection with any one serotype does not confer immunity against another. Even within a serotype distinct genetic and antigenic variants exist in different geographical regions with serious implications for the control of the disease by vaccination since it may render available vaccines inadequate.

In the 21st century FMD is still one of the most important livestock diseases due to its high infection rate (ease of spread) and its effect on the limitation of livestock movement and trade. The damaging effects of FMD on livestock production make the impact of the disease economically important and debilitating. FMD not only affects national and international trade, but impacts on the whole livestock industry with direct losses that result in damaging consequences for local farmers with invariable loss of income. FMD affects most of the major livestock animals of importance, i.e. bovidae (cattle, zebus, domestic buffaloes, yaks), sheep, goats and swine, in both high intensity farming systems and also in lower producing, developing countries. Although mortality is usually low (less than 5%), morbidity can reach 100% and cause severe losses in production, hence FMD is considered as the single biggest global threat to trade in livestock and livestock products in FMD-free countries. Therefore the effective control of FMD through vaccination, quarantine or slaughter-out

procedures are of paramount importance as it has financial implications world-wide.

FMD is widespread in Asia, India, Africa and certain countries of South America. The epidemiology of FMD in Africa is influenced by two different patterns i.e. a cycle involving wildlife and a cycle that is independent of wildlife but maintained within cattle. In the wildlife cycle, FMDV are maintained within African buffalo (*Syncerus caffer*) populations, the most common host of FMDV. These animals provide a potential source of infection for domestic livestock, like cattle, and other wildlife. Cattle may become persistently infected (carrier status) and circumstantial evidence indicates that carriers are able to transmit the infection to susceptible animals with which they come in close contact with. Elsewhere in the world cattle are usually the main reservoir, although in some instances the viruses involved appear to be specifically adapted to domestic pigs or sheep and goats. Wildlife outside Africa has not, so far, been shown to be able to maintain FMDV.

The main threat to areas free of FMD is the immediate consequences on trade in animals and animal products and the subsequent indirect losses through movement restriction of the human population from areas where the disease is present or suspected. The direct losses associated with disease control and re-emergence of disease into FMD clean areas through destruction of all affected or contact animals or through vaccination are also very high.

In areas endemically infected (most of Africa and regions of Asia, Latin America and Eastern Europe) the impact of the disease is not only associated with loss of trading opportunities but also the direct effect on the productivity of the animals through losses associated with milk yield, abortion, death in young animals and loss of traction power. Africa for example is endowed with an abundance of wildlife which in many instances has been well protected within national parks and game reserves. In communities neighbouring these parks, the livestock/wildlife interface presents unique challenges to livestock disease control. In addition, the ongoing creation of transfrontier conservation areas in Southern and Eastern Africa presents a particular challenge to the management of FMD

because they render the livestock/wildlife interface increasingly intense and complex. As a consequence, more flexible ways of managing FMD are required to obviate clashes between conservation-based and livestock-based initiatives aimed at rural development.

In endemic regions, the lack of infrastructure, human resources, movement controls and vaccines tailored to their conditions render many developing countries particularly vulnerable to the spread and poor control of FMD. Very often, livestock is raised under the communal smallholder systems and contribute to the livelihoods of the world's poor, especially vulnerable groups such as women and children. Animal diseases, like FMD, severely constrain livestock enterprises in developing countries. Crop farmers that rely on working cattle for ploughing are also affected due to loss of working power during an outbreak, affecting food security for the farmer and also for the country in question if the outbreak coincides with important crop activities. In many developing countries, vaccination will continue to be an essential component for the progressive control of FMD. Maximizing the effectiveness of current vaccines and supporting research to improve the effectiveness and quality of those and the development of new vaccines will be critical.

All these research activities are currently carried out by institutions members of the Global Foot and Mouth Disease Research Alliance (GFRA) and/or included in the FMD-DISCONVAC project funded by the European Commission within the 7th Framework Programme for Research and Technological Development.

The GFRA is constituted by 32 institutions, public and private, distributed in five continents. Many of the activities described here represent collaborative efforts between two or more GFRA partners. The vision and mission of the GFRA are concentrated in (i) coordinating a global alliance of scientists producing scientific evidence and innovation on FMD research, and (ii) establishing and sustaining global research partnerships in order to generate scientific knowledge and discover the tools to successfully prevent, control and eradicate FMD (http://www.ars.usda.gov/gfra/).

Several GFRA research programs are currently active in Europe, North America, South-East Asia, Australia, South America and South Africa. GFRA

programs will continue to expand the alliance in these regions and will actively reach out to new areas of the world that have a stake in the progressive control and eradication of FMD.

This report reflects activities performed towards the first two goals of the GFRA: (1) facilitate research collaborations and serve as a communication gateway for the global FMD research community and (2) conduct strategic research to better understand FMD.

The FMD-DISCONVAC project is funded by the European Commission within the 7th Framework Programme for Research and Technological Development. The project is structured through six research work-packages comprising vaccine-quality assessment, heterologous protection, vaccine development, diagnostics, transmission and development of computerized FMD spread models. The consortium involves 14 partners, mainly public institutions, but also private companies and laboratory networks. Most of them belong to the European Union but the Consortium also includes partners from Israel, Argentina, China and India. The Veterinary and Agrochemical Research Center (VAR, CODA-CERVA) from Belgium, holds the coordination of this project (http://fmddisconvac.net/).

The report is organized by major areas of interest and it is aimed to provide a global vision of the active programs and research areas on FMD, including, in some cases, brief descriptions of the results obtained so far. Furthermore it is envisaged to identify the gaps in strategic collaborations and research that may potentially prevent the progressive control and eradication of FMD in the future.

# Contributor Institutions

- Agence Nationale de Sécurité Sanitaire de l'Alimentation (AFSSA), Paris, France
- Agricultural Research Council-Onderstepoort Veterinary Institute (ARC-OVI), South Africa
- Agricultural Research Services, Plum Island Animal Disease Center (ARS-PIADC), Greenport, USA
- Center for Animal Disease Modeling and Surveillance (CADMS), UCAL-Davis, USA
- Central Veterinary Institute (CVI), Lelystad, The Netherlands
- Centro de Biología Molecular "Severo Ochoa" (CBMSO), Madrid, Spain
- CODA-CERVA, Veterinary and Agrochemical Research Center (VAR), Brussels, Belgium
- Commonwealth Scientific and Industrial Research Organisation, Australian Animal Health Laboratory (CSIRO-AAHL), Geelong, Australia
- Friedrich-Loeffler-Institut (FLI), Reims, Germany
- Indian Immunologicals Ltd, Hyderabad, India
- Institute for Animal Health (IAH), Pirbright, UK
- Institute of Virology and Immunoprophylaxis (IVI), Mittelhäusern, Switzerland
- Instituto Nacional de Tecnología Agropecuaria (INTA), Buenos Aires, Argentina
- International Livestock Research Institute (ILRI), Nairobi, Kenya
- Istituto Zooprofilattico Sperimentale Lombardia ed Emilia-Romagna (IZSLER), Brescia, Italy
- National Centre for Foreign Animal Disease (NCFAD), Winnipeg, Canada
- Ohio State University (OSU), Columbus, USA
- Red Interinstitucional de Investigación y Desarrollo en Fiebre Aftosa (RIIDFA), Argentina
- Technical University of Denmark, National Veterinary Institute (DTU), Lindholm, Denmark
- University of Glasgow (UGLA), Glasgow, Scotland

# Diagnostics and Vaccine Quality Control

***Developing new diagnostics tests and reagents***

a) Lateral flow devices: Lateral flow devices for pen-side testing have been developed, validated and commercialized for the detection of the viruses of foot-and-mouth and swine vesicular diseases. They can be used both in countries without easy access to laboratories and, in the event of outbreaks occurring in previously disease-free countries, for confirmation of secondary outbreaks. The IAH, in collaboration with colleagues from *IZSLER* and *Svanova Biotech AB* and the AAHL are working on this subject.

An immunochromatographic strip test for the rapid detection of foot-and-mouth disease viruses is currently being developed against all FMDV seven serotypes. The capture MAbs were conjugated with biotin and the detection MAb was conjugated with the colloidal gold particles. The preliminary results demonstrated that the strip tests are serotype specific. In order to increase the sensitivity, large sized gold particles (40nm and 60 nm) will be used to conjugate with the detection MAb (NCFAD).

b) ELISA-based assays: An FMDV antigen ELISA using integrin αvβ6 recombinant protein and monoclonal antibodies has recently been validated and offers increased specificity over the routinely employed polyclonal antibody based ELISA (IAH).

An improved IgA ELISA has been validated for the detection of carriers after use of the vaccinate-to-live policy (IAH within the FMD-DISCONVAC). Also, a multiplex immunoassays based on Luminex technology is being optimized (AFSSA within the FMD-DISCONVAC).

Conditions for stabilization of FMDV diagnostic reagents were investigated and stability for up to two years was demonstrated. Prototypes of ready-to-use kits for detection of antibodies specific to serotypes O and Asia 1 are under evaluation. Simple, rapid and stable ELISA kits for the diagnosis and typing of FMDV types O, A, Asia 1 and C were also developed (IZSLER within the FMD-DISCONVAC).

Pilot assessment of commercial kits for detection of antibodies induced against the non-structural proteins at ARC-OVI indicated that these tests, derived from the classical “European” types (A, O and C), may not be

sufficiently sensitive in areas where the SAT types predominate. Researchers at the ARC-OVI are therefore involved in the development of an improved NSP ELISA for Southern Africa. The test is currently being validated for commercial use in the SADC region in collaboration with CODA-CERVA-VAR and IZSLER Institutes and financial support from the FAO.

c) Real-time assays and genome sequencing: New real-time RT-PCR protocols has also developed and validated for the detection of FMDV. These assays have been adopted by the OIE and included into the Manual of Diagnostic Tests and Vaccines for Terrestrial Animals. During the 2007 outbreaks in the UK, data generated were supplied to government as part of the "Contain, control and eradicate" campaign. Furthermore, during the later stages of these outbreaks, the real-time RT-PCR assay supported an active surveillance program within high-risk cattle herds and reduced the un-necessary slaughter of uninfected animals. The IAH in a Defra funded work was at the lead of this project.

The method for sequencing the P1 viral capsid protein has been optimized at the NCFAD and three serotype O and five serotype A isolates available were analyzed and more will be sequenced. Genetic sequencing of FMDV capsid protein region will be used for strain differentiation (NCFAD in collaboration with IAH and the North American FMD Vaccine Bank)

d) Monoclonal antibodies and monovalent reference sera: Cell lines producing monoclonal antibodies (MAbs) specific for bovine cells are been produced and characterized, determining their viability and the presence of foreign animal diseases in vivo. Once cleared, the cell lines will be made available to colleagues in FMD free countries and/or deposited in the American Tissue Culture Collection for general distribution. This project is carried out by ARS-PIADC and ILRI in Nairobi, Kenya.

Eight neutralizing MAbs for FMDV serotype O were selected (five of them serotype O specific) for the characterization and selection of vaccine strains. In addition, polyclonal sera were prepared from rabbits, cows and guinea pigs against FMDV O 1 Manisa and O1 BFS. The antigenic relationship (r) of these virus isolates based on ELISA results and their ability to neutralize vaccine strains is being performed (NCFAD in collaboration with IAH and the North American FMD Vaccine Bank)

### *Harmonization of diagnostic tests*

One important goal is to obtain an equivalency of diagnostic test results for FMD and other related diseases among laboratories, regardless of protocols practiced. The activities of these programs are focused on sharing of reagents, training and developing of workshops for harmonization of tests.

Two members of the GFRA (NCFAD and ARS) participate in a collaborative program within this area together with the CPA in Mexico. NCFAD supported training and supply of reagents to CPA for AgELISA for FMD and VSV and for the FMDV 3ABC cELISA. During the Annual Workshop in 2010 organized by NCFAD, the following diagnostic tests were considered harmonized: FMDV rRT-PCR; FMDV AgELISA; FMDV VNT between USA and Canada; FMDV isolation between USA and Canada**.** All three countries are testing their VSV ELISA in 2011 and results will be compared. For SVDV harmonization, the results obtained from the Pirbright panels will be compared, after which the way forward will be discussed.

The standardization of FMD antibody response and protection is a key to harmonize several tests. The CVI in Lelystad has been working for several years on the use of a system with units of antibody based on a standard serum can decrease the variability between laboratories.

The FMD reference centers at ARC-OVI and Botswana Vaccine Institute (BVI) collaborated to harmonize the LPBE SOP for SADC. The harmonized SOP was used in a training workshop conducted at BVI during July 2010 and funded by FAO. Each participating country needed to set up the test in their laboratories with BVI providing all reagents. BVI conducted a proficiency test in October 2010 to determine the competency of the laboratories.

### *Vaccine quality control*

A collaborative project between USDA-PIADC and INTA seeks the identification of the genetic basis of animals with high and low responder phenotypes, which is accomplished through the determination of the heritability of the response to FMDV vaccination with commercial vaccine in naive cattle populations.

A research group at INTA has extensively tested murine models as an alternative to the use of cattle for vaccine potency assessment. Protection against podal generalization (PGP) and assessment of post vaccination antibodies by LPB-ELISA are the official tests in Argentina for potency control of FMD vaccines. Experimental and commercial FMD oil vaccines formulated with different antigen concentrations of inactivated FMDV were inoculated in cattle and mice and antibody titers at 60 (cattle) or 21 dpv (mice) were measured. Statistical association between antibody levels in mice and cattle was established thus indicating the feasibility of this approach that may and overcome the disadvantages of costs and facility needs derived from the use of large animals.

A filter-assisted luminometric ELISA to detect NSP contaminations in vaccine antigen preparations was developed in a collaborative project between ICT Cesar Milstein-CONICET, Argentina and Prionics, Lelystad. The proof of principle demonstrated the capacity of the system to quantify a non-structural protein (3ABC) at very low levels (up to 2 ng in 1 liter of filtrated preparation) in vaccine antigen batches from several manufacturers, regardless of their volume or composition, even in PEG concentrates. The final goal of this approach is to replace *in vivo* purity testing by determining the amount of 3ABC that induce a detectable immune response that may interfere with DIVA testing. This system is commercially available.

The FMD-DISCONVAC project also seeks to develop alternative *in vitro* assays to replace animal testing. The in vivo 50% Protective Dose (PD50) test is the standard European procedure for FMD vaccine potency testing in Europe. Due to ethical reasons, the VAR focuses on the replacement of the in vivo viral challenge by in vitro alternatives. Previously, an in vitro model for the FMDV reference strain O1 Manisa was validated. In a current study the VAR aims at developing a comparable model for serotype A and to test both models for serotype-independence. Tests with the potential to analyze the immunological activity of non-neutralizing antibodies are also being developed with the aim to apply them to FMD vaccine testing. Based on the observation that FMDV immune complexes efficiently interact with macrophages and that plasmacytoid dendritic cells result in the destruction of the virus or in IFN type I responses, Fc receptor-based assays to identify

the immunological activity of such antibodies are under development (IVI). Western Blot assays to detect the presence of NSPs in commercial vaccines and antigenic payload quantification and capsid integrity assessment methods based on chromatography were also developed (RIIDFA, Argentina).

# Epidemiology

### *Models*

One of the most relevant developments in FMD control is the application of computer simulation modeling to assess, predict and mitigate FMD outbreaks. The creation of a network of epidemiologist becomes fundamental to achieve the desired results. Transmission is one of the key components of the model and has been particularly evaluated together with the related environmental and spatial factors analysis. Other relevant aspects of the modeling are the animal movement, human factors related to animal disease incidence and persistence and the genetic sequence analysis of field isolates to identify carrier animals, viral determinants and viral evolution. In this case, the complete genome sequencing of the virus became a very informative tool and can be used in real-time to support epidemiological investigations. Moreover, with the new sequencing methods individual virus variants within a host can be identified. There is also, the possibility of simulating outbreaks with information recovered from the past which allowed the assessment of different control strategies. A risk analysis of FMD emergency vaccination could be performed in different scenarios and sometimes, it could be mixed with economical models to add this issue to the outbreak response.

Compilation of extensive global disease (FMD) surveillance data to make it publically available on the FMD BioPortal to research partners is in progress in a project directed by ARS and UCDavis.

The modeling project NAADSM [North American Animal Disease Spread Model] 5.0 was improved to incorporate (i) partial herd immunity & (ii) markets identified as priority changes for South America FMD situation. Epidemiologists from all 11 South American countries will be able to use a modified β version NAADSM 5.0 as a tool to enhance FMD preparedness

and response. NCFAD has served as a key component for the project with more than 15 other institutions from North and South America.

The CVI in Lelystad developed a new FMD transmission model based on data from transmission studies performed the last 10 years together data from the outbreak in 2001. It can be used to simulate outbreaks in the Netherlands and to study different control strategies. The model was supplemented with an economic model for the selection of the best control strategy in various parts of the country. The outcome shows that in a densely populated livestock area emergency vaccination (2km) is the cheapest option, whereas in sparsely populated livestock areas in the Netherlands (still densely populated in comparison with other countries) only killing animals on infected premises in combination with well implemented stand-still of movement of susceptible animals is considered the cheapest option. The analysis further shows that due to a smaller outbreak size the number of undetected infected animals is approximately 20 times smaller when emergency vaccination is used. This result shows that the current penalty for countries using emergency vaccination to regain freedom of FMD is not based on a solid risk assessment and should be changed.

ARS-PIADC and INTA together with UC-Davis work in a project to study the molecular epidemiology of FMDV in South America through virus sequencing, development of algorithms and predictive models to better understand epidemiological features and transmission history, and the identification of those genomic sites which facilitate changes in epidemiological features during outbreaks.

The FMD-DISCONVAC project is also working on the applicability and feasibility of modifying existing simulation models (InterSpreadPlus model, Davis model, NAADS model and other models within the Consortium) for FMD spread to suit the exploration of vaccination strategies in the EU and other Western European countries where FMD is considered an exotic threat. Computerized FMD spread models developed within this project could enable the design of vaccination strategies for high-risk regions within countries belonging to this consortium and could be relevant step-

stones to model vaccination strategies for truly endemic regions of the world (DTU within the FMD-DISCONVAC).

IAH and the UK Meteorological Office have shown that there is good reproducibility of models used in several different countries for predicting airborne spread of FMDV but differing assumptions on viral excretion patterns from index cases cause variability in outputs.

***Transmission***

Most infections with FMDV in the field are probably caused by direct contact between infected animals or their transport vehicles. However, part of the transmission is most likely caused by humans moving between farms. To determine which secretion or excretion is causing the highest virus output we reviewed the literature. Analysis showed that the data on secretion and excretion are different between species. The highest amount of virus is often found in vesicular material, but the volume is small. To study the relation between excretion and transmission two animal experiments were performed, one without vaccination and a second with vaccination. In both experiments both indirect and direct transmission was studied. The data of these experiments have to be analyzed further (CVI)

The effect of vaccination in preventing FMD transmission through contact exposure to the virus is being studied by carefully designed FMD transmission experiments (CVI funded by the FMD –DISCONVAC). A newly developed infection model will be used to study the ability of the Asian buffalo to transmit FMDV infection and to investigate the efficacy of vaccination to prevent this. The role of wildlife in FMDV maintenance and transmission, and quantified knowledge on the presence of FMDV in viral secretions and excretions in different species will be studied as well. Knowledge on FMDV transmission between species and in recently vaccinated animals can be used to adapt and improve computerized FMD spread models to optimize FMD vaccination programs in free and endemic settings alike. The aim is to obtain previously unavailable quantified knowledge on FMDV transmission within and between different FMDV susceptible species in the period shortly after applying emergency vaccination, and to study transmission dynamics in real-time outbreak situations to set-up early warning systems for FMDV penetration.

In order to analyse the outcome of FMDV challenge experiments, the IAH has developed novel Bayesian methods to infer the unobserved latent, incubation and infectious periods and the implications of these periods for the control of disease. Mathematical models were then used to scale from the challenge experiments to predict spread within cattle herds and sheep flocks. This has included collection of detailed data on the contact rates between animals within a sheep flock. Also nasal carriage by personnel exposed to FMD affected animals has been quantified and related to the risk this poses for farm-farm spread of the virus.

The IAH has also performed a qualitative risk assessment for safety of deboned beef as a traded commodity in relation to the spread of FMD, concluding that the product cannot be considered inherently safe without disease control mitigations as well as experiments of quantification of viral particle in the nose. Also, experimental studies have determined the infectious period of FMDV in cattle is shorter (mean 1.7 days) than currently realized and animals are not infectious until, on average, 0.5 days after clinical signs appear. These results imply that controversial pre-emptive control measures may be unnecessary for FMD and other acute viral infections of livestock and humans, if clinical inspection would be able to alert control agencies the moment clinical disease becomes apparent.

Transmission and evolution studies of FMDV in livestock in the Lake Chad Basin are currently carried out between ARS, Ohio State University Disease Epidemiology and Modeling Laboratory and Centre D'Appui a la Recherche et au Pastoralisme (CARPA), Maroua in Cameroon. The goal of this collaboration is to produce FMD transmission models for livestock in the Lake Chad Basin of Africa. This includes modeling animal movements, zoonotic disease transmission, environmental and spatial analysis of the factors related to disease transmission, molecular evolution of viruses and human factors related to animal disease incidence and persistence. Genetic sequence analysis of field isolates to identify carrier animals, viral determinants and associated epidemiological information will also be conducted.

### *Full length sequencing*

Genetic sequencing of FMDV capsid protein region for strain differentiation has been optimized in order to obtain a more comprehensive characterization and typing of the field viral isolates from different host of all seven serotypes. This tool is widely used for different GFRA members such as IAH, DTU, INTA, NCFAD, AAHL and their South East Asia partners, and ARS-PIADC with their partners in Vietnam, India, Afghanistan and Pakistan in order to gain a better understanding of the transmission and spread dynamics and mechanisms.

IAH and the University of Glasgow utilized full genome sequence data to reconstruct transmission pathways at the level of farm-to-farm spread. These methods have been used retrospectively to analyze clinical samples collected from the 2001 FMD outbreak, where it was shown that nucleotide changes, which frequently occurred throughout the genome, were inherited by progeny viruses in a manner that enabled known patterns of spread of the virus to be recreated. Similar methods were used in real-time to support epidemiological investigations into the 2007 FMD outbreak in Surrey and Berkshire, where results predicted the existence of undisclosed infected premises prior to their discovery by serological surveillance. Using next-generation sequencing (NGS) performed on a Genome Analyzer platform (Illumina), a recent project has compared the viral populations within clinical material collected from infected animals. This approach reveals the fine polymorphic sub-structure of the viral population, from nucleotide variants present at just below 50% frequency to those present at fractions of 1% and beyond.

A joint effort involving ARS-PIADC, the AAHL and the Department of Animal Health, Ministry of Agriculture Rural Development of Vietnam was put in place to determine the molecular epidemiology of FMDV in local livestock in Vietnam including cattle, buffaloes and pigs to gain a better understanding of the transmission mechanism of FMDV from persistently infected to susceptible local livestock in a natural setting. The enhancement of strategies for identification of persistently infected animals using new technologies will also be performed.

The FMD reference centers at ARC-OVI and BVI, in collaboration with the SADC TADs project undertook to sample buffalo herds in Zambia, Malawi, Mozambique and Tanzania. The SADC TADs project intends sampling buffalo in different national parks within these countries over a period of 3 years to determine the current status of FMD virus strains circulating in the buffalo herds. During the period August- September 2010 buffalo as well as cattle at the park interface was sampled as follows: probing samples and sera were collected from 25 buffalo and 25 cattle from in and around the Kafue National Park, Lochnivar National Park (Zambia), Lengwe National Park (Malawi), Marromeu National Park (Mozambique) and Katavi National Park (Tanzania).

# Pathogenesis

### *Early events in infection*

The early events of FMDV infection in cattle subsequent to simulated natural exposure were described by PIADC. Results shown that during previremic steers, FMDV was most consistently localized to nasopharyngeal tissues, thereby indicating this region as the most important site of primary viral replication. The earliest site of microscopic localization of FMDV antigens was the lymphoid follicle-associated epithelium of the pharyngeal mucosa-associated lymphoid tissue of the nasopharynx at 6 hours infection, within cytokeratin-positive pharyngeal epithelial cells. Also, intraepithelial FMDV-negative, MHCII/CD11c-double-positive dendritic cells were present in close proximity to FMDV-positive cells. Onset of viremia coincided with marked increase of viral loads in pulmonary tissues and with substantial decrease of viral detection in nasopharyngeal tissues. These data indicate that subsequent to aerogenous exposure to FMDV, the temporally defined critical pathogenesis events involve (1) primary replication in epithelial cells of the pharyngeal MALT crypts and (2) subsequent widespread replication in pneumocytes in the lungs, which coincides with (3) the establishment of sustained viremia.

### *Viral persistence and viral evolution*

Persistence of non-replicating but infectious virus has been demonstrated in germinal centers of lymphoid tissue, the role of this persisting virus could be very important in the cycle of the infection and the carrier state. Persistence of non-replicating but infectious virus has been demonstrated in germinal centers of lymphoid tissue in the head and neck of cattle, sheep, pigs and African buffalo. These observations will be further extended to understand the role of this persisting virus in maintenance of long-term protective antibody responses and generation of virus variation and recombination (IAH).

Full genome sequencing will be used at the ARC-OVI to investigate the mechanisms of virus persistence and new virus introductions as well as viral recombination and carrier animals in the evolution of new strains. This will provide information on the evolution and spread of the virus, ranging from within an individual animal to the global molecular epidemiology. Complete genome sequencing of FMDV can also provide data on potential inter- and intratypic recombination, although the role of recombination in altering virus virulence, pathogenicity or host range is not yet known.

A CBMSO group has been working for years in quasispecies evolution, using Foot-and-Mouth Disease virus as a model system. In recent years the main interest of the group has been in exploring lethal mutagenesis (virus extinction by excess of mutations) using base and nucleotide analogues as mutagenic agents. The results have unveiled mechanisms of ribavirin resistance in FMDV mediated by alterations of the viral polymerase (3D) that either prevent incorporation of ribavirin into progeny RNA, or modify the recognition of ribavirin by the enzyme to keep a balance among different transition types, a balance that favors virus survival.

### *Pathogenicity and virulence*

Research seeking to describe and characterize new viral factors of pathogenicity and virulence of FMDV are also carried out at INTA funded by the ANPCyT (Argentinean National Agency for Science and Technology Promotion) and through a collaborative agreement with ARS-PIADC. Both projects are focused on two isolates (FMDV A/Arg/00 and FMDV A/Arg/01)

obtained during the 2000/2001 outbreaks in Argentina. These strains presented different clinical signs in cattle; these differences could also be reproduced in cell cultures and in animal models. In the first project, an infectious clone based on a natural isolate of VFA A/Arg/01 was developed and chimerical viruses containing different regions (IRES, S fragment and non structural protein regions) from FMDV A/Arg/00 in replacement of the homologous sequences were constructed. These chimeras will be used to study the effect of each region on FMDV virulence and pathogenicity. A second project will compare the pathogenic capacity of the FMD viruses strain A/Arg/01 isolated from cattle and FMD viruses from the same strain but derived from the corresponding infectious clone. The associated pathogeny will be studied for each virus and correlated with their corresponding quasispecies composition after serial passages in mice.

The ARS-PIADC and the National Veterinary Research and Quarantine Service of the Republic of Korea are studying the pathogenicity of FMDV Korean isolates, A/ROK/2010 and O/ROK/2010 in pigs and cattle. Focus is placed in time-course studies of FMD infection including direct transmission. A catalog of quality pictures of lesions and reference stocks of research material, serum and tissues, will be obtained.

DTU-Vet, Lindholm is also working on the analysis of virus determinants of replication in cells and host animals (e.g. characterization of the pathogenicity of chimeric viruses).

# Immunology

### *Early immune response*

Several lines of research are trying to unveil unknown aspects of the interaction between live and/or inactivated FMDV (vaccines) with different immune tissues and cell types in both susceptible species and experimental models. This information will become critical in the design of novel strategies for immunization and protection against natural infection. The early induction of local adaptive immune responses in the respiratory tract of infected cattle has been described through a collaborative project between ARS-PIADC and INTA. Experiments performed at INTA demonstrated that the onset of local FMDV-specific antibody responses is

at 4 days post-infection (dpi), with a strong stimulation of the tracheobronchial lymph nodes followed by mandibular and retropharyngeal lymph nodes. These results were in agreement with previous findings that related the onset of viremia with a marked increase of viral replication in pulmonary tissues (PIADC, see above). The FMDV-specific antibody secreting cells reached up to 0.4% of the total mononuclear cells isolated from the mandibular and other mucosal lymph nodes at 6 dpi, thus indicating the existence of a very robust local response in parallel with the systemic humoral response.

Furthermore, rapid induction of CD4 T cell-independent antibody responses and the formation of virus-antibody immune complexes (IC) have been identified as a key event in disease pathogenesis in cattle. IC formation triggers productive infection and apoptosis of dendritic cells (DC) and induction of type-1 interferon production from plasmacytoid DCs, events that correlate with induction of clinical signs and transmission (IAH).

In this same line, previous results obtained at INTA had demonstrated the interaction of the FMDV with DC in a murine model. Research is now conducted to study the impact of such interaction in the development of adaptive responses against FMDV in mice. Current results show that initially live virus produce a greater inhibition in spleen DC subsets than inactivated virus. However, after 24 h of FMDV infection, T lymphocyte proliferation is restored while vaccination increases the recruitment of plasmacytoid DCs and the induction of IL-10 that trigger the activation of regulatory T-cell responses.

A project focused on the study of mechanisms of early immune enhancement against FMDV is conducted by ARS-PIDCT and IAH laboratories. This project seeks to investigate the role of dendritic cells response to FMDV infection and in response to vaccination against FMDV in swine and cattle. Development of an alternative platform for vaccination will endeavor to stabilize the virus capsid in the vaccine construct thereby allowing rapidly induction of protective antibodies and cell mediated immune responses. When these stabilized empty capsids become available, these new vaccines will be added to the analysis of new recombinant vaccines.

CVI is conducting experiments to identify regions within the FMDV genome of the O NET 2001 strain responsible for the reduced blocking of type 1 IFN in culture cells. Type 1 IFN is part of the innate immune system and can play a role in the early defence of the host against viral infection. The reduced blocking of type 1 IFN made it difficult to grow the O NET 2001 virus in porcine kidney cell cultures during the 2001 epidemics, thus forcing the switch to ovine kidney cells. Infectious copies of the O NET 2001 and O Manisa virus were produced, together with a little more than 10 mutant viruses containing different parts of both genomes. These chimerical viruses will be tested for type 1 IFN induction on porcine kidney cells.

***Duration of immunity and cross-reaction between serotypes***

ARS-PIADC and DTU are engaged in a collaborative project aiming to improve FMDV vaccine potency and duration of immunity through the study of the cellular immune response to infection and the ability to refine the killed virus vaccine for FMDV or the recombinant empty capsid vaccine. Specific analysis of the T cell responses to FMDV infection in swine and cattle will be conducted; focusing on the identification and mapping of epitopes and the development of histocompatibilty complex tetramers which will be used to measure T cell response.

Also, in a BBSRC-funded project, an IAH research group has demonstrated that both FMDV infection and vaccination prime $CD8^+$ T cell responses. A conserved $CD8^+$ T cell epitope has been identified within the FMDV structural protein 1D which stimulates a cross-reactive response to seven serotypes.

ARS-PIADC and the Indian Veterinary Research Institute in Bangalore (IVRI) are conducting antigenic and genetic characterization of FMDV field virus isolates using the Ad5 platform developed in PIADC to understand FMD antigenic structure and cross protection. This research will help to gain a better understanding of FMD antigenic variation and vaccine coverage in support of FMD control programs in India.

# Vaccines and Antivirals

### *New antigens*

The IAH in collaboration with other institutes has produced a vaccine from a GH loop negative FMDV isolate which is capable of protecting animals from virus challenge. The constructs used in these studies have also demonstrated a consistent pattern of amino acid changes, lying on, or near, the pentameric interfaces of the capsid structure. These constructs are more thermostable and show greater pH resistance than wild-type virus.

A research group at the CBMSO is working in the development of new FMDV marker vaccines (peptides and DNA vaccines) that can induce protective humoral and cellular immune responses in pigs. They are also analyzing the functional role of FMDV non-structural proteins on the internalization, the replication cycle and the pathogenesis of FMDV and other related Picornaviruses in cell culture and animal models. A parallel study of the functional implications of non-coding RNA regions is conducted for the identification of antiviral targets and the design of new vaccine strategies. Their results showed that FMDV non-coding RNA fragments are potent inducers of type-I interferon in cultured cells and experiments are being performed to assess the potential antiviral effect of these RNAs *in vivo*.

The IAH has constructed and developed FMDV marker vaccines using recombinant Sendai virus (rSeV) and adenovirus. Expression of FMDV capsids in mammalian cell culture has been difficult due to the toxicity of FMDV 3C protease required for cleavage of the viral polyprotein. To mitigate the toxicity of FMDV 3C protease in cell culture, the natural gradient of transcription in paramyxoviruses has been exploited. Following this idea, the 3C coding sequence was inserted close to 5' end of the Sendai virus genome to reduce its level of expression and FMDV P1-2A was inserted the at the 3' end to produce a rSeV expressing FMDV capsids in mammalian cells. This project, funded by a commercial biotechnology company and the FMD-DISCONVAC, has great potential for the development of a mucosal vaccine against FMD.

Likewise, recombinant FMDV antigens vectored by human adenoviruses are under development. The aim of this project is to generate DC-targeted FMDV antigens that may be administered through the oronasal route to generate mucosal immune responses with improved specific T-cell stimulation (INTA funded by the ANPCyT)

Development of novel vaccines is also being progressed by studies of bovine afferent lymph DC (ALDC). In vitro comparisons of the interaction between viral vaccine vectors and ALDC populations successfully translated to enhance DC targeting of adenovirus vectored antigen with improved T cell responses.

Other studies for vaccine development are also conducted within the FMD-DISCONVAC project. The use of recombinant baculovirus as potential adjuvants for conventional inactivated vaccines has been tested in a murine model. Immunization and challenge experiments were performed in mice using formulations including inactivated FMDV O1 Campos and (pol-) AcNPV (BV). Early humoral and cytokine responses as well as protection to the homologous challenge were observed in this model. This strategy will be now tested in cattle using baculoviruses carrying additional bovine CpG motifs (INTA, see below). An expression plasmid containing the P1 and 3C FMDV coding sequences has been constructed and a recombinant EMCV with a deletion in the 2A coding region was generated and the recombinant cDNA genome was produced (AFSSA).

INTA is also working, funded by the ANPCyT, on basic studies about the adjuvant properties of baculoviruses. Preliminary results in mice showed that baculovirus have strong antiviral properties against FMDV, promoting early protection against FMDV A/Arg/2001 challenge in C57Bl/6 mice. This fact, together with the potent innate immunostimulating effects of baculovirus (mostly mediated by interaction of CpG motifs with TLR9), make them promising candidates for FMDV prevention. This project is focused on the evaluation of the underlying mechanisms that may allow recombinant baculovirus, bearing host-specific CpG motifs, preventing infection in natural hosts.

The ARC-OVI, together with PIADC and Intervet, have developed a reverse genetics and vaccine design approach to significantly improve vaccine

performance in the field by (1) producing vaccine antigen for specific geographic regions, (2) enhancing capsid stability and increasing duration of immunity, (3) improve cell-culture growth and antigen yield during production (O and SAT2 serotypes) and (4) modify antigenicity. Enhanced capsid stability will have a positive effect on thermal tolerance and extend the shelf-life of a vaccine. Currently, live recombinant viruses can be recovered and inactivated vaccine produced in the conventional manner. However, the feasibility of applying this technology on an industrial scale has not yet been tested. In an international collaborative research project funded by the Wellcome Trust, researchers at IAH, Oxford University, PIADC, ARC-OVI and Intervet will produce and test *in vivo* recombinant vaccine products in cattle to determine the efficacy of the structurally designed vaccines.

The ARC-OVI is currently in the process to establish techniques for adapting new SAT vaccine strains to cell culture without the need for porcine cells to prevent contamination of vaccine seed viruses. Isolates from recent FMD studies or outbreaks in the southern African region for SAT 1, 2 and 3 topotypes have been selected for this purpose. These viruses will be adapted on various cell cultures through passaging, due to the selective pressure of the virus to infect a specific cell line. Samples will be taken for titration and 146S determination at each passage to determine whether viruses are adapting and to eliminate poor seeds. This approach, in addition to the reverse genetics approach, is aimed to select improved vaccine strains for the various antigenic variants within the SAT serotypes found in southern Africa.

***Cross-Protection and Vaccine Matching***

A challenge for FMD vaccines is the existence of seven serotypes of the FMDV that are not cross-protective. Moreover, cross protection within serotypes is also limited for certain strains.

The FMD-DISCONVAC includes different vaccine matching projects using O and A serotype strains. The Friedrich-Loeffler-Institut (FLI) coordinates this WP, also harmonizing different in vitro assays to predict cross-protection within serotypes. In vivo cross-protection studies showed that serological cross-reactivity between serotype O viruses is not always a good indicator

of cross-protection. Increasing payload may be beneficial (Indian Immunologicals). Experiments were carried out with mono- and bivalent serotype A vaccines against field isolates within serotype A. In general r-values were improved if calculated on the basis of grouped sera and sera classified by titre (RIIDFA, Argentina). Alternative vaccine matching methods such as antigenic cartography and sequence based antigenic characterisation are under investigation (IAH and University of Glasgow).

Prospects for the development of a more cross-protective vaccine have been increased by EU-funded studies that show FMDV DNA prime/protein boost regimen in pigs not only conferred protection against FMDV but also induced an enhanced and cross serotype reactive neutralising antibody response. Subsequently, in BBSRC funded studies, five different DNA prime boost vaccination regimes, and particularly those involving an electroporation step, were capable of protecting cattle from a homologous virus challenge.

The CVI has conducted experiments together with veterinarians from Eritrea using a set of 10 type A antigens selected for immunisation of 5 cattle each. The 32 week post-vaccination sera have been used in neutralization tests, neutralization index and Liquid Phase Blocking ELISAs. Analysis of the results using different statistical techniques showed that each technique produces different results. This large set of data shows that there is no single best technique for this analysis and the outcome is always biased by the technique used.

Researchers at the ARC-OVI are also engaged in different collaborative projects aimed to develop indirect and informatics-based methods to select vaccine strains that match against field isolates, maximizing the immunological protection that can be induced. Several approaches were explored to define the viral epitopes that elicit protective B cell responses and using these antigenic determinants to predict or measure antigenic relatedness between emerging viruses and vaccine strain. In one approach, they combined structural and genetic data from the virus capsid proteins and in vitro cross-protection titres to predict those predictors of antigenicity. This is being done in collaboration with researchers at the University of Glasgow. In an alternative approach recombinant antibodies

panned from a phage-displayed antibody library were utilised to map antigenic regions on the virus capsid. The recombinant antibodies are also proposed to be used as reagents in screening contemporary viruses to determine the antigenic relatedness against existing vaccine strains. The latter project is being done in collaboration with PIADC.

Vaccine matching projects are also being conducted by RIIDFA institutions in Argentina. These experiments are performed using strains within the O serotype and results of the homologous and heterologous challenge assays will be correlated with different parameters of the specific humoral and cellular immune responses elicited after vaccination.

IAH is also working on the study of protective capacity of conventional and emergency vaccines. They have initially established, with Defra funding, that a single dose of emergency FMD A serotype vaccine is capable of maintaining a protective immune response for at least 6 months in cattle. A program of work in systems biology for FMDV has also been established, which to date includes: (i) understanding FMDV-induced lysis of bovine epithelium; and (ii) investigating the impact of vaccine stability on immunogenicity. The group has analyzed and interpreted large-scale serological surveys carried out in Jordan and Somalia and initiated new interdisciplinary studies in Nigeria, Cameroon and Mali.

***Antivirals***

Studies on antiviral development are also conducted within the FMD-DISCONVAC project. At the VAR more than 35.000 small molecules were screened in vitro for a potential inhibitory effect on FMDV replication on SK-6 cells. Antiviral activity was assessed by light microscopic evaluation of cytopathic effect (CPE) and by a resazurin-based colorimetric cell-viability assay. RNA loads were determined with two semi-quantitative real-time RT-PCR methods. Different molecules with panserotypic antiviral activity against FMDV at concentrations that did not have adverse effects on the SK-6 cells were identified. A hit explosion for the most potent inhibitors is currently ongoing. The in vitro antiviral activity of at least 3 of these compound families will be further improved through a hit-to-lead optimization program. Antiviral escape mutant viruses will be generated to study the molecular antiviral mechanism of action of these compound

families. Following optimization, a preliminary in vivo assessment of the antiviral activity will be performed in an FMDV infection model in severe combined immunodeficient mice (VAR).A research group at INTA is also engaged in developing new antiviral strategies based on artificial microRNA. Three target regions of the FMDV genome were selected and transgenic cell lines constitutively expressing one or multiple artificial microRNAs against them were established. Whereas some of these cell lines proved to efficiently silence a reporter gene fused to the FMDV target sequence, replication of an FMDV-A infectious clone in transgenic cell lines was not impaired. Ongoing experiments are trying to determine the role of FMDV RNA secondary and tertiary structure in the accessibility of putative artificial microRNA target sequences.

# Molecular Biology of the Infection

### *Replication of the virus*

The FMDV-receptor interaction is under study by IAH and Surrey University, funded by BBSRC. The initial interaction of FMDV with its principle receptor (integrin αvβ6) is cation-dependent, but on binding, a highly stable, EDTA-resistant complex, rapidly forms. The complex stability of the integrin αvβ6 and the virus is dependent on a helical structure immediately C-terminal to the RGD and two conserved residues at positions RGD+1 and RGD+4. An ability to induce such stable complexes with αvβ6 is likely to contribute significantly to the high infectiousness of FMDV. Further studies have shown that FMDV infects three-dimensional, porcine nasal mucosal and tracheal mucosal epithelial cell cultures predominantly using integrin αvβ6 to initiate infection. Once inside the cell, FMDV infection (i.e. membrane penetration) takes place predominantly from within early-endosomes and does not require virus trafficking to late-endosomal compartments.

CBMSO is currently working in how the IRES governs protein synthesis. IRES elements operate as ribonucleoprotein complexes in which RNA structure and IRES function is tightly coupled. Conserved structural elements have been identified that are required for FMDV IRES activity determining tertiary interactions. The functional role of FMDV non-structural proteins is analyzed in cell culture and animal models. It has been found that FMDV

non-coding RNA fragments are potent inducers of type-I interferon in cultured cells and experiments are being performed to asses the potential antiviral effect in vivo of these RNAs. Additionally, isolated novel IRES-interacting proteins that form part of regulatory networks of gene expression had been identified.

Murine models for the picornavirus, human rhinovirus have been developed at IAH; demonstration of membrane permeability by rhinovirus capsid protein VP4; assembly of FMDV capsid pentamers; characterization of the picornavirus, equine rhinitis A virus, as a model for FMDV, including structure and uncoating, receptor interactions and endocytic pathway. Also, with collaborators at Oxford, they have developed a real-time fluorescent assays for measuring picornavirus particle stability and genome release.

***Structural Studies***

The CBMSO and the DTU are interested in the study of the molecular determinants of assembly and stability of viral particles, and applications for the design of vaccines and antivirals. The CBMSO group uses three models: FMDV, MVM and HIV-1. The FMDV structure has been engineered to obtain virus particles with increased thermostability. This group has obtained modified virions that are normally infectious but that show dramatically improved thermostability, thus being suitable for the development of non-cold chain dependent vaccines. They are also exploring virus stabilization mechanisms, inhibition of viral processes and compensating mutations.

In the structural design of improved recombinant vaccine in the control of FMD, research groups from IAH, Oxford, ARC and PIADC has looked at residues in the structural proteins of the virion that may contribute to the stability of the virion in various environmental conditions and yield in cell culture. These residues are currently being investigated using infectious genome-length clones for there respective roles in the virion.

INTA has also started research aimed to explore the structural interaction between the main antigenic site of the virus (the G-H loop) and the variable region of selected monoclonal antibodies.

# Other Research and Support Programs

1) Enhanced diagnostic capability in South America

Two GFRA members, the NCAD from Canada and the ARS-PIADC from USA, have leading roles in a collaborative project seeking the enhancement of FMD preparedness by transferring technical methodology and knowledge either by meetings or courses. The project is composed of two sub-projects: a. Enhanced diagnostic capability in the Andean region of South America and b) Application of computer simulation modeling to assess, predict and mitigate FMD outbreaks. In this project, NCFAD has served as a key component in providing training, protocols, and reference reagents for FMDV isolation and rRT-PCR for rapid diagnosis to national reference laboratories for FMD in Andean countries. The packages of techniques include virus isolation, antigen ELISA typing, vaccine matching and real-time RT-PCR. These projects are being carried out together with PANAFTOSA PAHO/WHO, LIDIVET (Bolivia), Laboratorio Nacional de Diagnóstico Veterinario CEISA (Colombia), Laboratorios de Sanidad Animal - AGROCALIDAD (Ecuador), Unidad del Centro de Diagnostico de Sanidad Animal Laboratorio de Enfermedades Vesiculares (Perú), Ministerio del Poder Popular para la Agricultura y Tierras, (Venezuela) and the IICA.

2) Support programs in Pakistan

The ARS-PIADC and CADMS, UC-Davis are collaborating with Pakistan laboratories to: (i) characterize local isolates of FMDV and development of vector based vaccines, (ii) apply epidemiological models to understand the emergence of new FMDV antigenic and genetic variants and (iii) strength laboratory capacity and vaccine matching activities in Pakistan by providing real time epidemiological support tools to help the formulation, implementation, and evaluation of progress of the FMD control program in Pakistan implemented through FAO

3) Programs in Australia and South East Asia

FMD is endemic in certain parts of the world and occurs in many countries in South East Asia (SEA), through its proximity the biggest perceived risk to Australia's agricultural economy. For this reason industry and the federal Australian government are funding a project focusing on aspects of FMD

such as protection of various cloven-hoofed species using the vaccine strains in the bank, pathogenesis of SEA viruses in equivalent Australian domestic species, field validation of pen-side assays, molecular epidemiology of FMD in SEA and capacity building in the region as part of our pre-border mitigation.

The funding is provided through Meat and Livestock Australia's Donor Company and will initially be for 2 years with an expectation of an extension for 3 more years. The contracts have only recently been signed and there is no scientific progress to report to date.

Since no live FMD virus is allowed into Australia, all the animal challenges will have to be done offshore in collaboration with GFRA and other partners. The pig challenges will be performed in Vietnam, the sheep challenges in South Africa at the BSL3 facility at the Onderstepoort Veterinary Institute. Cattle will be challenged at the new facility for SENASA (The National Animal Health and Agri-food Quality Service) in Argentina. In addition, experts from two GFRA partners (CVI and USDA-PIADC).

As a contingency for live virus work, Australian officials are collaborating with the FMD Regional Reference Lab based in Pakchong, Thailand, where significant capital and other investments in the lab will be provided to perform the high throughput work.

# Research Gaps

The following list has been mainly build based on the conclusions presented in a report produced for the US National Veterinary Stockpile after the FMD Gap Analysis Workshop conducted by a group of international researchers on FMD in Buenos Aires, August 2010.

**Diagnostics**

- New technologies for pen-side testing
- Evaluation and validation of commercially available pen-side tests to "fit for purpose" for surveillance, response, and recovery
- Proof-of-concept of herd immunity tests correlating with efficacy of vaccines in the vaccine banks.
- Identify FMDV-specific non-structural protein antigenic determinants for development of DIVA diagnostic tests
- Develop serotype specific rRT-PCR assay(s)
- Assess the use of air sampling technologies and validate their use for FMDV aerosol detection in open and enclosed spaces.

**Epidemiology**

- A global FMD surveillance system that provides high quality, accurate, and real-time information on FMD risk is needed to cover critical gaps of information of the FMD situation worldwide and to support FMD control and eradication on a global scale;
- Epidemiological models should be applied to identify key areas of the world to be targeted for active collection of samples and information, and for monitoring the evolution of the disease as part of the global FMD surveillance system in critical regions of the world;
- Training on epidemiological analysis has to be promoted in endemic regions of the world to pursue control of the disease at a global scale
- Analytical tools to support the decision making process has to be developed, including, a) anomaly detection methods to identify outlier events; b) 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; c) 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

- Sensitivity and specificity of diagnostic tests and surveillance systems have to be evaluated at global, regional, and national scales.

**Viral Pathogenesis and Transmission**

- Identify determinants of viral virulence for different serotypes of FMDV in cattle, sheep, and swine.
- Investigate virus-host interactions at the primary sites of infection in ruminants and their role in determining infection.
- Investigating the wildlife-livestock interface as an important factor in FMD control.
- Determine the early events in FMDV pathogenesis in swine and small ruminants (i.e., primary site of replication, mechanisms of spread)
- Development of a reproducible FMDV challenge method in swine
- Determine FMDV immune evasion mechanisms
- Determine mechanisms of FMDV persistence in livestock and its role in transmission

**Immunology**

- Study mucosal responses to acute and persistent infections in cattle
- Establish the immune mechanisms underlying protection to FMDV during the time-course of infection
- Study neonatal immune responses to infection and vaccination and the influence of maternal immunity in protection and vaccine efficacy
- Determine the role of cellular innate immune responses in FMDV infection of cattle and swine and the correlation between cellular immune responses and vaccine efficacy and protection.
- Develop methods to activate cells of the innate response to anti-viral activity (NK cells, γδ T cells, and DCs)

- Contract the development of antibodies to surface markers of critical immune bovine and porcine cell types as well as specific for bovine IFN-α and β as well as porcine IFN-β

**Vaccines and Antivirals**

- Understand and overcome the barrier of serotype- and subtype-specific vaccine protection (achieve cross-protection and/or increasing the breadth of antigenic coverage)
- Improve available FMD vaccines investigating key issues such as obtaining increased antigen yields, stability of vaccine antigens, enhanced vaccine-induced immunity and vaccination frequency.
- Invest in the discovery of new adjuvants and immune modulators to improve the efficacy and safety of current inactivated FMD vaccines.
- Studies to characterise FMDV capsid structures such as epitope mapping to assist in better understanding of the immune responses evoked in animals and enhanced design of vaccines.
- Develop vaccine formulations effective in neonatal animals with or without maternal immunity
- Develop vaccinal needle-free strategies to induce mucosal as well as systemic responses in susceptible species
- Develop vaccine formulations and delivery targeting the mucosal immune responses
- Investigate the safety and efficacy characteristics of novel attenuated FMD vaccine platforms (e.g. leaderless FMDV)
- Develop next generation FMD vaccines that prevent FMDV persistence
- Testing Ad5-IFN distribution and expression in cattle after aerosol exposure.
- Evaluate the ability of GenVec Ad-type I IFN platform to confer rapid onset of protection (18 hr) against several FMD serotypes and subtypes

## Cross-Protection and Vaccine Matching

- Support research on the immunological mechanisms of cross protection in susceptible species to understand and overcome the barrier of serotype- and subtype-specific vaccine protection (achieve cross-protection and/or increasing the breadth of antigenic coverage)
- Continuous vaccine matching in different regions/countries (especially for type A and SAT2).
- Increased knowledge and understanding of the correlation between *in vitro* serological and *in vivo* cross-protection tests. Development of new non-*in vivo* strategies to predict cross protection

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