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

Report: October 2011

Update of Research Activities

FAO-EUFMD

Research Activities Worldwide

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

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Contents

Purpose of the Report

The following report compiles the updated information (6-month update of annual report, 2010) on current research activities provided by multiple contributors from FMD research laboratories distributed around the world. This report highlights new or ongoing projects within the various FMD research areas of interest, in addition to the projects described in the 2010 annual report. Consequently, this document should be used as providing a general guide to active research topics on FMD around the world, but it should not be expected to be fully comprehensive or consistent in its level of detail.

The Global Foot-and-mouth Disease Research Alliance (GFRA) aims to expand FMD research collaborations worldwide and maximize the use of resources and expertise to achieve its five strategic goals:

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

This 2011 update report reflects current (2011) 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. In some instances the research progress made in 2011 has also been captured. 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. Furthermore, it is envisaged that the report will be used to identify the gaps in strategic collaborations and research that may potentially prevent the progressive control and eradication of FMD in the future.

1. BACKGROUND AND INTRODUCTION TO THE REPORT

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 and 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 is 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.

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/).

2. DIAGNOSTICS AND VACCINE QUALITY CONTROL

2.1 Developing new diagnostics tests and reagents

The Annual Report (2010) described the development, validation and/or commercialization of lateral flow devices (IAH, *IZSLER, Svanova Biotech AB and* AAHL) and MAb-based immunochromatographic strip tests (NCFAD) for the detection of FMDV. The development and/or validation of ELISA-based assays have been described, including (i) an integrin αvβ6 recombinant protein-based ELISA (IAH), (ii) an improved IgA ELISA (IAH within the FMD-DISCONVAC), (iii) optimized Luminex technology-based multiplex immunoassays (AFSSA within the FMD-DISCONVAC), and (iii) a NSP-based ELISA with improved sensitivity for the detection of antibodies to the SAT types (ARC-OVI, collaborative testing and validation at CODA-CERVA-VAR and IZSLER institutes, FAO funded). New validated real-time RT-PCR protocols for the detection of FMDV have also been incorporated into the OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (IAH, Defra funded). The protocol for P1 viral capsid protein sequence determination has been optimized (NCFAD) and the sequences of several serotype O and A isolates were analyzed. The production of cell lines producing MAbs,

specific for bovine cells, has received attention (ARS-PIADC and ILRI) and serotype O-specific MAbs for vaccine strain characterization and selection were identified. Polyclonal antisera were prepared in small animals and cows against FMDV O 1 Manisa and O1 BF and the antigenic relationship (r) of these virus isolates were investigated (NCFAD, IAH and the North American FMD Vaccine Bank). The subsequent sections describe a number of the current research activities that are directed towards the development of FMD diagnostic tests and reagents.

(a) Immunoassays for the detection of FMDV

A collaborative initiative between ARC-OVI, CODA-CERVA-VAR, IZSLER Institutes, investigated a SAT-specific ELISA test to determine the diagnostic accuracy (sensitivity and specificity) of the assay before it can be applied as a diagnostic tool in southern Africa. Although the statistical analysis still need to be finalized, the preliminary results suggest that the SAT-specific ELISA performed similarly to two other validated NSP-based ELISA assays which enable discrimination between cattle either infected with SAT viruses, or vaccinated with SAT vaccines. This project that came to an end in October has been financially supported by the FAO in SADC.

The principle goal of a project entitled “Rapid Diagnostics for the Field (RAPIDIA-FIELD)” is to develop ready-to-use, rapid field diagnostic kits for infectious animal diseases (Funding: EU/FP7). The development of AlphaLisa (AFSSA) for direct detection of FMDV using antigen capture with antibodies is linked to this proposal. The main objectives of the RAPIDIA-FIELD project proposal are (i) to improve sample collection and preparation, (ii) to develop simple, cost-effective serological tests for direct multiple pathogen detection in the field that could be used by minimally trained personnel, (iii) to develop simple laboratory methods that allow a sensitive, specific and multiple detection of antibodies and pathogens, and (iv) to confirm and reference the techniques. Adaptation of the new assays for use in different species (including wild life) or in the preparation of reference samples will also receive attention. Tools and approaches that are developed in the RAPIDIA-FIELD project will be made available to laboratories and authorities that are interested in applying the technology to diagnose and control infectious veterinary diseases. Collaborators include: (1) Inmunología y Genética Aplicada, S.A. (INGENASA); (2) Friedrich-Loeffer-Institut (FLI); (3) PRIONICS; (4) PROPHYL Ltd; (5) Instituto Nacional de Técnica Aeroespacial (INTA); (6) Institute for Animal Health, Pirbright (IAH); (7) French Agency for Food, Environmental and Occupational Health and Safety (ANSES); (8) Universidad Complutense Madrid (VISAVET); (9) CODA-CERVA; (10) Swedish Uni. of Agricultural Sciences, National Veterinary Institute (SVA); (11) IDEXX Switzerland AG; and (12) OPTIGENE.

(b) Immunoassays for the detection of antibodies against FMDV

Currently, a multiplex immunoassay that allows simultaneous detection of antibodies to multiple viral antigens in a single reaction is being developed and evaluated (AFSSA). The aim of this research is to develop a multiplex immunoassay for FMD diagnosis by using the Luminex liquid array technology. This assay will allow, in a single reaction and from a single sample, simultaneous detection of antibodies against vesicular disease viruses and FMDV structural and non-structural proteins, coupled to microspheres labeled with different proportions of fluorescent dye. Such a test will thus allow differential diagnosis of FMD but also differentiation between infected and vaccinated animals. This activity is performed within the FMD-DISCONVAC project that comprises different, yet interlinked, work packages and is funded by the European Commission within the 7th Framework Programme for Research and Technological Development (EU/FP7). The following institutions are contributing to the development of the multiplex immunoassay: (1) Centrum voor Onderzoek in Diergeneeskunde en Agrochemie (CODA-VAR); (2) Institute for Animal Health (IAH); (3) Stichting Dienst Landbouwkundig Onderzoek part, Centraal Veterinair Instituut (CVI); (4) Istituto Zooprofilattico Sperimentale della Lombardia e dell´Emilia Romagna (IZSLER); (5) Friedrich Loeffler Institut, Bundesforschungsinstitut fuer Tiergesundheit (FLI); (6) Indian Immunologicals Limited (IIL); (7) Lanzhou Veterinary Research Institute (LVRI); (8) Fundaciòn para la interacciòn de los sistemas productuvo, educativo, científico-tecnológico (FUNPRECIT); (9) Agence Nationale de Sécurité Alimentaire (ANSES); (10) Eidgenossiches Volkswirtschafts-departement, Institute of Virology and Immunoprophylaxis (IVI); (11) Ministry of Agriculture, Kimron Veterinary Institute (KVI); (12) University of Glasgow (UGLA); (13) Danmarks Teknikse Universitet (DTU) and (14) Merial S.A.S. (MERIAL).

The development and evaluation of a multiplex immunoassay that allows simultaneous detection of antibodies to multiple viral antigens in a single reaction will also be investigated at the AFSSA as part of the RAPIDIA-FIELD proposal, the details of which were described in Section 2.1 (a).

(c) Molecular assays for the detection of FMDV

A multiplex molecular assay, that allows simultaneous detection of multiple viruses in a single reaction, is currently being developed (AFSSA). The principle aim is to develop a suspension microarray based on Luminex^{TM} technology that will enable molecular detection of the seven serotypes of FMDV, as well as differential diagnosis from other vesicular diseases (SVDV, VSV and VESV). The activity is linked to a project entitled “AniBioThreat: Bio-preparedness measures concerning prevention, detection and response to animalbio-threats” which aims to improve the European Union’s (EU) capacity to counter biological animal threats in terms of awareness, prevention and contingency. The focus of the project will be based on threats to

living animals, animal feed and food of animal origin. As part of this, it is foreseen that the project will enhance international cooperation and promote networking for bridging security with animal and public health. The objectives are based upon some of the identified actions in the Commissions EU CBRN Action Plan. Financial support towards the development of the multiplex molecular assay is provided by EU/DG Justice and the research is performed in collaboration with the: (1) French Agency for Food, Environmental and Occupational Health and Safety (ANSES) in France, (2) Swedish University of Agricultural Sciences, National Veterinary Institute (SVA) in Sweden; (3) Danmarks Teknikse Universitet (DTU) in Denmark; (4) Netherlands National Institute for Public Health and the Environment (RIVM) in Netherlands; (5) Central Veterinary Institute (CVI) in Netherlands; (6) Istituto Superiore di Sanità (Superior Health Institute) (*ISS*) in Italy; (7) Istituto Zooprofilattico Sperimentale delle *Venezie* (IZS) in Italy; (8) Central Agricultural Office, Directorate of Veterinary Medicinal Products (CAO-DVMP) in Hungary and (9) Swedish University of Agricultural Sciences in Sweden.

The DTU is working on different research activities in collaboration with the IAH, National Veterinary Laboratory in Pakistan, Imperial College in the UK and Makarere University in Uganda. These activities can be segregated into each of the six research areas and is mentioned in this report under the respective headings. Funding is provided from internal, as well as from the Danish Research Council, the Danish Development Agency (Danida) and EUFMD (FAO). With regard to molecular assays, the DTU is working on the development of serotype-specific real-time qRT-PCR assays, designed on the basis of knowledge of the sequences of circulating virus strains.

2.2 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 organization of workshops aimed at the harmonization of tests. Towards this end, the Annual Report (2010) outlined that NCFAD and ARS participated in a collaborative program within this area together with the CPA in Mexico, and several assays were regarded as being harmonized between USA and Canada. In 2010, it was also reported that the FMD reference centers located at the ARC-OVI and the Botswana Vaccine Institute (BVI) collaborated to harmonize the LPBE SOP for SADC and the training workshop was funded by FAO. The standardization of FMD antibody response and protection is a key to harmonize several tests. Consequently, in order to decrease the variability between laboratories, the CVI in Lelystad has an ongoing project focusing on the use of a system with units of antibody based on a standard serum.

2.3 Vaccine quality control

The research projects described in the Annual Report (2010) concerning the GFRA research activities is continuing into 2011. In short, these included: (1) a collaborative project between USDA-PIADC and INTA which aims to identify the genetic basis of animals with high and low responder phenotypes by determination of the heritability of the response to FMDV vaccination with commercial vaccine in naive cattle populations; (2) extensive testing murine models (INTA) as an alternative to the use of cattle for vaccine potency assessment; (3) development of a filter-assisted luminometric ELISA to detect NSP contaminations in vaccine antigen preparations (ICT Cesar Milstein-CONICET, Argentina and Prionics, Lelystad) and (4) the FMD-DISCONVAC project which seeks to develop alternative *in vitro* assays to replace animal testing. No new research projects aimed at the improvement of vaccine quality control have been reported.

3. EPIDEMIOLOGY

3.1 Models

Several research projects aiming at the development and application of computer simulation modeling to assess, predict and mitigate FMD outbreaks have been described in the 2010 Annual Report of the GFRA Research Activities and are ongoing. Briefly, these projects included: (1) the compilation of an extensive global disease (FMD) surveillance database to be made publically available on the FMD BioPortal to research partners (ARS and UC-Davis); (2) the modeling project, NAADSM, developed by NCFAD in collaboration with more than 15 other institutions from North and South America; (3) a new FMD transmission model developed by CVI in Lelystad; (4) the development of predictive models to better understand epidemiological features and transmission history by ARS-PIADC, INTA and UC-Davis and (5) the FMD-DISCONVAC project focusing on the applicability and feasibility of modifying existing simulation models (InterSpreadPlus model, UC-Davis model, NAADS model and other models within the Consortium) to suit the exploration of vaccination strategies in the EU and other Western European countries.

3.2 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. The 2010 Annual Report of the GFRA research activities extensively described several ongoing studies by GFRA member institutions. These transmission and evolution studies, continuing into 2011, is being performed by the (1) Nethertlands' Central Veterinary Institute (CVI) in Lelystad; (2) Institute for Animal Health (IAH)

in UK; (3) ARS in Ohio State; (4) University Disease Epidemiology and Modeling Laboratory and (5) Centre D'Appui a la Recherche et au Pastoralisme (CARPA) in Cameroon.

In addition, a new collaborative research project between IAH, ARC-OVI and DAFF set forth to investigate direct transmission between African buffalo (*Syncerus caffer*) and cattle in a natural setting (Section 4.1). Four buffalo experimentally infected with FMD and confirmed as carriers by the presence of viral RNA in oropharyngeal fluid, are being housed with a domestic cows in separate clean pens from day 35 post-infection. The cattle are being continuously inspected for clinical signs of FMD. Extensive tissue sample will be collected when clinical signs of FMD appear or at the end of the experiment.

3.4 Sequencing and phylogenetic analysis of FMD viruses

Nucleotide sequencing of FMDV capsid protein coding-region are continuing being used for comprehensive characterization and typing of emerging and re-emerging field viral isolates. This tool is widely used by different GFRA members including the IAH, DTU, INTA, NCFAD, ARC, 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 epidemiology and spread dynamics of the disease.

A joint effort between the ARS-PIADC, AAHL and the Department of Animal Health, Ministry of Agriculture Rural Development of Vietnam was initiated in 2010 to determine the molecular epidemiology of FMDV in local livestock in Vietnam. Surveillance of cattle, buffaloes and pigs is included in order to gain a better understanding of the transmission mechanism of FMDV from persistently infected to susceptible local livestock in a natural setting. The project is ongoing and also includes the enhancement of strategies for identification of persistently infected animals using new technologies.

The FMD reference centers at the 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 to sample 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 2010 and 2011, buffalo, as well as cattle at the park interface was sampled as follows: probang 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).

FMD is endemic in Pakistan and Afghanistan and FMDV serotypes O, A and Asia-1 are responsible for outbreaks and diverse strains, even within the same serotype, co-circulate. Characterization circulating FMD viruses can facilitate suitable vaccine selection and tracing of outbreaks. The FMDV strains that have been circulating in Pakistan and Afghanistan in the past few years were characterized by nucleotide sequencing and evolutionary analysis was performed. The results of these studies (National Veterinary Laboratory, DTU and Quaid-i-Azam University in Pakistan) are in press or have been published (Section 12) as described below.

In the first study, the nucleotide coding sequences for the VP1 capsid protein or for all four capsid proteins (P1 region) of the serotype A viruses circulating in Pakistan and Afghanistan were determined using numerous representative samples collected between 2002 and 2009 (Jamal *et al*., 2011a). Phylogenetic analysis of the VP1 coding sequences revealed the presence of at least four lineages within two distinct genotypes, all belonging to the Asia topotype, within serotype A. The predominant lineage (A-Iran05) is actively evolving at a high rate and seven distinct variants were identified, the dominant being the A-Iran05AFG-07 and A-Iran05BAR-08 sub-lineages. The A22/Iraq FMDV vaccine is antigenically distinct from the A-Iran05BAR-08 viruses. Surface-exposed amino acid differences between the capsids of these viruses that may explain the antigenic difference were identified.

In the second study, the genetic diversity of FMD serotype O viruses that circulated in Pakistan and Afghanistan between 1997-2009 have also been determined (Jamal *et al*., 2011c) and revealed the presence of at least three different lineages (Pak98, Iran2001 and PanAsia) within the ME-SA (Middle East South Asia) topotype. The PanAsia lineage is currently dominant in the area and is actively evolving giving rise to new variants, as revealed by the appearance of distinct variants e.g. PanAsia-II, and a new variant designated here as PanAsia-III.

The third publication (Jamal *et al*., 2011b) describes phylogenetic analysis of FMDV type Asia-1 and the results indicated that between 1998 and 2009, three different genetic groups (II, VI and a new group, designated Group VII) have circulated in Pakistan, while viruses from Groups I and -II have circulated in Afghanistan. Using near complete genome sequences, from FMD viruses of serotypes Asia-1 and A that are currently circulating in Pakistan, an inter-serotypic recombinant virus was identified, which has the VP2-VP3-VP1-2A coding sequences derived from a Group-VII Asia-1 virus and the remainder of the genome from a serotype A virus of the A-Iran05(AFG-07) sub-lineage. The Asia-1/Shamir vaccine strain is antigenically distinct from the serotype Asia-1 viruses currently circulating in Pakistan and Afghanistan and, therefore, new Asia-1 vaccine strains may be required to block the spread of the current Asia-1 viruses.

Lastly, the genetic diversity of FMDV serotype C in Kenya (East Africa) was evaluated (Makerere University in Uganda, DTU) and evidence for probable vaccine strain re-introductions in the field was obtained (Sangula *et al.*, 2011).

4. PATHOGENESIS

4.1 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 (IAH). 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). During a pilot study the IAH, ARC-OVI and the Kruger National Park Veterinary Services (KNP-VS, Department of Agriculture, Forestry and Fisheries of DAFF) have detected FMDV genome at these sites in buffalo within the Kruger National Park.

Therefore, the IAH, ARC-OVI and DAFF set forth to investigate the hypothesis that lymphoid tissue is the site of FMDV persistence and FMDV variants accumulate on follicular dendritic cells in germinal centres in buffalo. Retention of intact FMDV particles on the follicular dendritic cell network provides a mechanism for maintaining a highly cytopathic and lytic virus-like FMDV extracellularly. Intermittent shedding of variants from this site to the periphery could result in low-level virus replication and transmission. For FMDV, it has been shown that multiple infections of cells can occur *in vitro* resulting in recombination. Persistently infected buffalo can maintain more than one type of SAT virus simultaneously, with a potential for recombination if a susceptible cell takes up more than one serotype or subtype. The project aim is to determine how FDMV is maintained and how antigenic diversity is generated in African buffalo.

The CBMSO has been working for years in quasispecies evolution, using FMDV as a model system. Their continued research project and recent progress has been summarized in the 2010 Annual Report.

Significant discrepancies are found in reports regarding the pathogenesis of FMDV infection in cattle with specific emphasis on the anatomical sites involved in early and persistent virus replication. The sites of virus replication within cattle and the nature of the host response to virus infection, including the induction of interferon and acute phase proteins, have been investigated in detail (DTU) and the results of these studies are being published. The presence of FMDV RNA was demonstrated in pharyngeal epithelium biopsy samples obtained from infected cattle (Stenfeldt and Belsham, in press). Whereas, low levels of FMDV RNA were

present in samples of pharyngeal epithelia during both early and persistent phases of infection, significantly higher levels of virus were detected in pharyngeal excretions. Therefore, the targeted area for sampling within the DSP does not harbor significant levels of virus replication during acute or persistent FMDV infection in cattle. In addition, the DSP and the mandibular and retropharyngeal lymph nodes cannot be concluded to be principal sites for persistence of FMDV.

4.3 Pathogenicity and virulence

The status of projects described in the 2010 Annual Report of the GFRA Research Activities is unchanged in 2011. The two projects include: (1) a collaborative research project between INTA and ARS-PIADC, funded by the ANPCyT (Argentinean National Agency for Science and Technology Promotion) seeking to describe and characterize new viral factors of pathogenicity and virulence of FMDV and (2) investigation of the pathogenicity of FMDV Korean isolates in pigs and cattle (ARS-PIADC and National Veterinary Research and Quarantine Service of the Republic of Korea).

The DTU have performed targeted modification of full-length virus cDNAs, produced chimeric viruses and evaluated the pathogenicity of these chimeric viruses that have been rescued from the cloned cDNAs. The results of this study have been published recently. The chimeric viruses comprised the capsid coding sequences derived from the O/UKG/34/2001 or A/Turkey 2/2006 field viruses inserted into a cDNA backbone derived from the parental strain (O1K B64). Compared to the rescued parental O1K B64 virus, the chimeric viruses grew well in primary bovine thyroid cells, but less efficiently in BHK cells. The two chimeric viruses displayed the expected antigenicity in serotype-specific antigen ELISAs. Whereas sufficient attenuation of the rescued parental was confirmed, inoculation of cattle with each chimeric virus resulted in the development of clinical signs of FMD, which then spread to in-contact animals. Consequently, there is no evidence for any adaptation, acquired during cell culture, outside the capsid coding region within the O1K B64 strain that inhibits replication in cattle. These chimeric infectious cDNA plasmids provide a basis for the analysis of FMDV pathogenicity and characterization of receptor utilization *in vivo*.

In addition, FMD viruses that are pathogenic for cattle (Pakistan and Afghanistan) have been rescued from preserved viral RNA samples (DTU) and the results have been published this year (Section 12). A system that has been developed to rescue infectious FMDV from RNA preparations, generated from clinical samples obtained under experimental conditions, were applied to samples collected in the "field". Following collection, the samples were treated to preserve the RNA and were then transported to DTU. FMDV RNA extraction and quantification with real-time RT-PCR samples, containing significant levels of RNA, were introduced into

susceptible cells. Progeny viruses were amplified in primary bovine thyroid cells and characterized by making use of an ELISA, RT-PCR and sequencing. FMD viruses of three different serotypes and multiple lineages have been rescued from the RNA samples, after which two (serotype O and Asia 1) were inoculated into bull calves under high containment conditions. Acute clinical disease was observed in each case which spread rapidly from the inoculated calves to in-contact animals. Thus the rescued viruses were highly pathogenic. The availability of the rescued viruses enabled serotyping by antigen ELISA and facilitated genome sequencing. In conclusion, the procedure described here should improve the characterization of FMDVs circulating in countries where the disease is endemic and thus enhances disease control globally.

In early 2011, FMD was found in wild boar in Bulgaria near the Turkish border, followed by two series of outbreaks in domestic animals in the adjacent region. To date, wild boar are not considered an important reservoir for FMD virus and only one well documented infection experiment in this species has been published (Rodriguez-Calvo et al., 2011). Therefore, a transmission experiment with the 2011 Bulgarian FMDV type O isolate was conducted at the FLI Riems in wild boar and domestic pigs. The most striking result of the experiment was the discrepancy in the clinical course between domestic pigs (severe clinical FMDV) and wild boar (mild clinical course) while wild boar shed large amounts of virus for several days. The clinical, virological and serological data generated in this experiment will help to understand and forecast FMD in wild boar populations, which is of extreme importance for regions with high densities of wild boar e.g. huge parts of Europe.

5. IMMUNOLOGY

5.1 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 the ARS-PIADC and INTA. IAH demonstrated a CD4 T cell-independent antibody response and the formation of virus-antibody immune complexes (IC) as a key event in disease pathogenesis in cattle. 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. In addition, the 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. These projects have been extensively described in the 2010 Annual Report of GFRA Research Activities.

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

The characterization of the immune response of cattle to FMDV forms part of a research program at the DTU and modulation of cytokine mRNA expression in pharyngeal epithelial samples obtained from FMDV-infected was recently demonstrated (Stenfeldt *et al.*, in press). Expression of IFN-β mRNA was significantly down-regulated in the biopsy samples harvested during the acute phase of infection, while there was no statistically significant effect on the expression of IFN-α mRNA compared with baseline levels. On the contrary, the mRNA encoding TNF-α was significantly up-regulated in samples collected during both acute and late phases of infection. Furthermore, there significantly higher levels of TNF-α mRNA were expressed in samples derived from animals that were identified subsequently as persistently infected FMDV-carriers. It was concluded that there was a significant difference in the host-response in the DSP of calves that were identified as persistently infected, subclinical carriers of FMDV.

The acute phase responses of Serum Amyloid A, Haptoglobin and Type 1 Interferon in cattle experimentally infected with FMDV serotype O and possible implications for the development of persistently infected "carriers" have been investigated (Stenfeldt *et al.*, in press). The results indicated a significant increase in serum concentrations of both APPs and type 1 IFN in infected animals coinciding with the onset of viraemia and clinical disease. Furthermore, the results suggest that there was no systemically measurable inflammatory reaction related to the carrier state of FMD. There was a statistically significant difference in the HP response between carriers and non-carriers with a lower response in the animals that subsequently developed into FMDV carriers. It was concluded that the induction of SAA, HP and type 1 IFN in serum can be used as markers of acute infection by FMDV in cattle.

5.2 Duration of immunity and cross-reaction between serotypes

The 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. Furthermore, in a BBSRC-funded project, the IAH 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. The 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 at the 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.

6. VACCINES EFFICACY AND NEW GENERATION VACCINES

6.1 Vaccine efficacy

FMD is of major concern to the Australian livestock industries, prompting the respective government and these industries to fund the compilation of a FMD vaccine bank for use in the event of an outbreak. However, there has been little investigation into vaccine efficacy against infection with a heterologous FMD virus strain. FMD occurs in many countries in South East Asia (SEA) and is perceived as the biggest risk to Australia's agricultural economy, due to the geographical proximity of these countries. Consequently, a project (AAHL) is funded by the Meat and Livestock Australia (through funding from the Australian livestock industries and federal government) focusing on several aspects of FMD. The aims of this project include (i) protection of various cloven-hoofed species using the FMDV strains in the vaccine bank, (ii) pathogenesis of SEA viruses in equivalent Australian domestic species, (iii) field validation of pen-side assays, (iv) molecular epidemiology of FMD in SEA (Section 3.4) and (v) capacity building in the region as part of their pre-border mitigation. Since no live FMD virus is allowed to enter Australia, all the animal challenges have to be performed offshore in collaboration with the GFRA and other partners. The pig challenges will be performed at the NAVETCO and RAHO6 in Vietnam, while the sheep challenges will be performed in South Africa at the BSL3 facility at the ARC-OVI. Cattle will be challenged at the new facility of SENASA (The National Animal Health and Agri-food Quality Service) in Argentina.

6.2 New antigens

(a) Production of FMD viruses with increased stability

The IAH, in collaboration with the Oxford University, PIADC, ARC-OVI and Intervet, is working on the production of structurally modified master seed viruses to enhance the conventional FMD

vaccine production (Funding: Wellcome Trust). New serotype O and SAT2 viruses, with enhanced stability at 49°C, were produced and are being prepared for formulation to carry out comparative immunization studies in Guinea pigs. The development of rapid and accurate methods for the quantification of FMDV particle stability, through the transfer of technology from Oxford University to IAH, was included in the proposal. Different assays for the quantification of FMDV stability of wild-type and mutated particles (serotype O and/or SAT2) were used, i.e. the conventional method of measuring capsids after ultra-centrifugation in sucrose gradients, an infectivity assay, a novel assay that was developed at Oxford University, and an ELISA-based assay that detects dissociated pentamers. With the conventional method, a 10-fold enhancement in the stability of mutated serotype O and SAT2 virus particles was measured. Using the novel assay (Oxford University), the stability of wild-type and mutated serotype O viruses enabled detection of wild-type capsid dissociation at 43°C, while the mutated serotype O virus only dissociated at 58°C. Noteworthy, one alternative benchmark that the researchers set was to produce serotype O virus particles with the same stability as serotype A virus particles. In the novel assay, serotype A virus particles dissociated at 56°C. With the ELISA-based method, a clear difference in the magnitude of detectable pentamers between wild-type and mutated serotype O capsids at 49°C were observed, while pentamers could not be detected in the mutated virus samples, suggesting no dissociation at 49°C. Whereas for the wild-type virus the dissociated pentamer signal was equivalent to the total viral protein signal, which is consistent with the results of the novel assay (Oxford University), quantification of pentamers and total viral particle protein using the ELISA is still being validated. A mutated SAT2 infectious virus was constructed (ARC-OVI) based on structural information provided by the Oxford University which displays increased thermostability. The ELISA-based assay (IAH) can not be utilized, because the antibodies fail to bind to SAT2 viruses. Consequently, an infectivity inactivation assay has been utilized to measure virus stability. The results of this study indicated a ten-fold increase in the stability of the mutant SAT2 virus at 49°C. For improved quantification of the stability of SAT-2 virus particles, the novel assay has also been transferred from Oxford University to the ARC-OVI and is currently being established there.

The *in silico* simulations used to identify structural modifications to the viruses have developed rapidly and the results of the above experimental studies using the mutated viruses (IAH) suggest the models are performing well. In addition to using these models to design new vaccine viruses, they can potentially be used to help select which field strains are likely to produce the most stable vaccines based on their predicted structure. Identification of mutations to accelerate cell culture adaptation is also progressing well. A combination of sequence analysis of viruses before and after cell culture adaptation, as well as model predictions has been used to produce mutated viruses. The capacity of these viruses to grow in cell culture is being assessed. Furthermore, second generation mutations are under development. Mutations

have been successfully introduced into a number of serotype O viruses to facilitate growth in cell culture and vaccine production.

(b) Reverse genetics vaccines

In a newly initiated research collaboration (USDA-ARS-PIADC, Makerere University in Uganda and ARC-OVI), entitled "Research and Development of Counter Measures against FMDV in Uganda", it is envisaged that newer and more effective molecular vaccines will be developed for use in endemic countries including Uganda. In doing so, detailed antigenic and genetic characterization of FMD field virus isolates, surveillance, and monitoring and vaccine strain selection for FMD control programs in Uganda will be performed. Additionally, this collaborative research project will also contribute to the mission of the GFRA, an international initiative, which supports and coordinates FMD research with the goal of controlling and eradicating FMD worldwide. Among the major constraints to livestock productivity in Uganda are (i) inadequate water sources; (ii) diseases, (iii) inadequate feed and (iv) a poor market for livestock products. FMD is perhaps the most important disease and the main trade barrier affecting the marketing of livestock products. Serotypes A, O, C, SAT1, SAT2 and SAT3 are known to circulate in Uganda, causing disease in wildlife and domestic animals. The main problems are the high morbidity and serious impact on growth and production, resulting in the disruption of trade and a decrease in market value of livestock products. Most importantly, there is a need to improve surveillance and detection of FMD, as well as to characterize FMDV virus strains and to perform effective vaccine matching in order facilitate suitable vaccine selection. Currently, Uganda imports inactivated FMDV vaccines. However, the use of these vaccines in Uganda is complicated by the vast genetic and antigenic variability characteristic of especially serotypes A, SAT1 and SAT2 FMD viruses, and the continuous selection of mutants from the population escaping the host's immune response. Therefore, there is a need for rapid sequence and antigenic profiling of viruses as part of the FMD control program in the region. Only in recent years and in collaboration with European FMD laboratories, an *in house* method of seven serotype-specific solid-phase blocking ELISAs (SPBE) has been implemented for FMDV diagnostics in Uganda. The damaging effects of FMD on livestock production make the impact of the disease economically important and debilitating. For these reasons, it is one of the most feared livestock diseases and a major reason why Uganda has failed to gain access to export markets.

In view of the above, it is necessary to ease the problems of the farmers by setting up strategies for effective surveillance and vaccination of animals against FMD. Production of adequate FMD vaccines that match the circulating strains in Uganda is an imperative as part of a strategy for the progressive control of FMD in Uganda. Therefore, this research collaboration between the USDA, Makerere University (Uganda) and ARC-OVI aims to: (1) develop the necessary tools

and monitoring systems in support of surveillance, and the progressive control and future eradication of FMD in Uganda; (2) perform FMDV field strain collection in support of the surveillance program in Uganda or in certain regions (University of Makerere and Ugandan government Veterinary Services) and includes characterization of circulating FMD viruses, their distribution in Uganda, and antigenic vaccine matching studies, (3) develop FMD vaccine strains tailored to the needs of Uganda by cloning the relevant FMDV capsid-coding regions of the Uganda strains into well characterized, cloned FMDV cDNA backbones, and (4) to evaluate the potential vaccines and compare their protective efficacy with that of the commercially available vaccines.

(c) Production of empty FMD virus capsids

The IAH, in collaboration with the Oxford and Reading Universities, is working on the production and evaluation of FMDV stabilized capsids as potent, rapidly deployable vaccines (funding: FAO-EUFMD) and, thus far, excellent progress has been made. A DNA “expression cassette” was generated that can be exchanged between different expression systems to produce empty FMDV capsids in mammalian or insect cells. Several expression systems have been assessed and the most useful for screening and scale up have been identified. Proof of principle experiments have been performed to demonstrate the use of recombinant technology to produce FMDV empty capsid vaccines with improved physical stability. Current research activities will focus on the optimization of commercially viable expression systems, including baculovirus, yeast and mammalian expression systems and selection of the best system for optimum yields and cost-effective production. All of the major animal health companies have been informed of this research and a patent application has been submitted to protect the novel technology that has been developed. One of the aims is to optimize FMDV empty capsid production (serotypes A, Asia, O and SAT2) using the vaccinia virus dual-infection system of mammalian cells by further refinement of capsid sequences (IAH and Oxford University). Towards achieving this goal, empty capsids have been produced for serotypes A, O and SAT, while DNA constructs for serotype Asia have been made and are currently undergoing expression trials. Serotype A capsids, derived from either a wild-type or a mutated sequence shown to greatly enhance capsid physical stability, have been produced in sufficient quantities and purity for crystals structures to be solved. This is a very important development, because it is known that the synthetic capsids are very similar in structure to the virus, hence will share protective antigens. The structure of serotype O capsids, derived from wild-type sequence, has been solved and the antigenic structure was found to closely resemble that of the native virus. Towards achieving the production of stable FMDV capsids *in vitro* using the baculovirus expression vector system, stable serotype A and O capsids have been produced (IAH, Oxford University and Reading University). The yield of the serotype A virus capsids (ca. ≤1ug/ml) are consistent with the average yield obtained for the current (conventional) FMD vaccine

manufactured in the commercial production facilities. The IAH and Oxford University are also currently investigating alternative methods to express FMDV capsids. Researchers have managed to produce O and A serotype virus capsids in *Trichoplusia ni* larvae. This expression system has been used extensively to produce reagents for diagnostic tests and may be an alternative protein expression platform for vaccine production. It is certainly possible to produce large quantities of proteins using this method. These evaluations are ongoing. The IAH is furthermore developing methods to quantify antigenic mass in vaccine candidates and evaluate the efficacy of novel vaccination regimes. Single-chain lama-derived antibodies that specifically recognise 146S and 12S capsid fragments are available at IAH and they have developed ELISA-based assays to quantify serotype O antigen. In addition, the capacity to detect 12S fragments of all serotypes has allowed the development of ELISA-based assays to determine the thermostability of the synthetic capsids. In summary, empty capsids with greatly enhanced physical stability have been produced for A serotype viruses in sufficient quantities to be commercially viable. Cattle have also been immunized with a dose of antigen consistent with commercial vaccines, delivered in a commercial adjuvant. Protective levels of neutralizing antibody were induced and maintained for over 5 months post-vaccination. These animals will be challenged to confirm protection. Capsids for other serotypes are under development and it is envisaged that a full portfolio of protective vaccines to be available in the near future.

As part of a collaborative research program (Section 2.1 c), the DTU is also developing systems for the expression of specific regions of the FMDV coding sequence to generate empty capsid particles as vaccine candidates.

(c) Development recombinant FMD vaccines using viral vectors

Two activities relating to recombinant vaccine development are currently being conducted at AFSSA within the FMD-DISCONVAC project. Firstly, the development of recombinant FMD vaccines by making use of canine adenoviruses (Cav) that will be safe for use in animals is under investigation. The CaV vector vaccine, expressing the FMDV VP1 protein or co-expressing multiple FMDV capsid proteins will be evaluated in mice and pigs. Secondly, AFSSA is working on the development of recombinant FMD vaccines by making use of an attenuated encephalomyocarditis virus (EMCV) expressing all the capsid proteins of FMDV. The efficacy and safety of the candidate EMCV vector vaccine will be evaluated in mice and pigs.

The IAH is also performing viral vector studies. Targeting dendritic cells (DC) is the key to driving effective immune responses. Lymphatic cannulation provides access to the heterogeneous populations of DC draining peripheral sites in rodents and ruminantş. Afferent lymph DEC-205$^+$CD11c$^+$SIRPα$^+$ DC were preferentially infected *ex vivo* with three vaccine viral vectors: replication defective human adenovirus-5 (rhuAdV5); modified vaccinia virus Ankara

(rMVA) and fowlpox virus (rFPV), all expressing green fluorescent protein (GFP). The rhuAdV5-infected cells remained viable and peak GFP expression observed 16-24 h post-transduction. Increasing the incubation period of DC with rhuAdV5 enhanced GFP expression. In contrast, DC-infected with rMVA-GFP or rFPV-GFP became rapidly apoptotic and GFP expression peaked at 6 h post-infection. Delivery of FMDV A_{22} antigen to DC by rhuAdV5-FMDV-A_{22} *ex vivo* resulted in significantly greater $CD4^+$ T cell proliferation compared to rFPV-FMDV-A_{22}. Delivery of rhuAdV5-GFP in oil adjuvant *in vivo*, to enhance DC-vector contact, resulted in increased GFP expression in migrating DC compared to vector alone. Similarly, $CD4^+$ T cell responses were significantly enhanced when using rhuAdV5-FMDV-A_{22} in adjuvant.

6.2 Cross-Protection and Vaccine Matching

FMD vaccine development is complicated by not only the existence of seven serotypes of the FMDV, which are not cross-protective, but also the variation that occurs within a serotype. Distinct antigenic variants exist in different geographic regions with implications for the control of the disease through preventative vaccination, since it may render available vaccines less effective. Several institutes and collaborative ventures embrace vaccine matching projects as part of their research. The FMD-DISCONVAC program includes different vaccine matching projects based on serotype O and A FMDV strains. The Friedrich-Loeffler-Institut (FLI) coordinates this work package, also harmonizing different *in vitro* assays to predict cross-protection within serotypes. At the FLI, cattle challenge experiments were carried out according to the European Pharmacopoeia to assess the protection induced by A Iran 05 and A22 vaccines against recent A Iran 05 topotype virus isolates from the Middle East viruses. It was concluded that the A Iran 05 vaccine is a valuable component of European vaccine banks, but may not protect against some recent isolates. It is attempted to correlate the challenge results with in-vitro results like r-values und neutralization titres. The results of *in vivo* cross-protection studies indicated 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 characterization are under investigation (IAH and University of Glasgow).

The results of studies towards the development of a more cross-protective candidate vaccine have (EU-funded) show that a FMDV DNA prime/protein boost regimen in pigs not only conferred protection against FMDV, but also induced an enhanced and cross-serotype reactive neutralizing 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 ten serotype A antigens selected for immunization of five cattle each. The 32-week post-vaccination sera have been used in neutralization tests, neutralization index and Liquid Phase Blocking ELISAs (LPBE). Different statistical techniques were used to evaluate the data and comparative analysis revealed 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 with the Glasgow University, IAH and SACIDS, aiming 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 to use these antigenic determinants to predict or measure antigenic relatedness between emerging viruses and vaccine strains. In one approach, structural and genetic data from the virus capsid proteins, and *in vitro* cross-protection titres was combined to predict the indicators of antigenicity. This work 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 utilized 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 serotype O 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.

The IAH is also working on the study of protective capacity of conventional and emergency vaccines. They have initially established that a single dose of emergency FMD serotype A vaccine is capable of maintaining a protective immune response for at least 6 months in cattle (Defra funding). A program which focuses on systems biology for FMDV has also been established and the primary aims are (i) to understand FMDV-induced lysis of bovine epithelium and (ii) to investigate 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.

7. MOLECULAR BIOLOGY OF THE INFECTION

7.1 Replication of the virus

The role of individual FMDV proteins in virus replication is currently under investigation by DTU and their collaborators (see Section 2.1 c).

The FMDV-receptor interaction is under investigation by the IAH and Surrey University, and the project is 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 motif 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.

The CBMSO is currently investigating the mechanism IRES-driven 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 were analyzed in cell culture and animal models. The results indicated 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 these RNAs *in vivo*. Additionally, isolated novel IRES-interacting proteins that form part of regulatory networks of gene expression had been identified.

7.2 Structural Studies

In the 2010 Annual Report, the CBMSO and DTU expressed their interest in studying the molecular determinants of assembly and stability of viral particles, and applications for the design of vaccines and antivirals. This research is ongoing. Three models are utilized i.e., FMDV, MVM and HIV-1. The FMDV structure has been engineered to obtain mutated virus particles with increased thermostability which are, thus, ideal for the development of non-cold chain dependent vaccines. They are also exploring virus stabilization mechanisms, inhibition of viral processes and compensating mutations.

Within the Wellcome Trust funded project, described in Section 6.1, the IAH, Oxford University, ARS-PIADC, ARC-OVI and Intervet are investigating the link between thermostable FMD

antigens and longevity of the immune response. 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 were identified. These residues are currently being investigated using various infectious genome-length clones for there respective roles in the virion stability.

The INTA has also initiated a research project which aims 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.

11. GFRA INSTITUTIONS

MEMBERS	ASSOCIATES	COLLABORATORS
CSIRO's Australian Animal Health Laboratory (AAHL), Australia	Central Veterinary Research Laboratories, Department of Virology, FMD Unit, Sudan	Center for Animal Disease Modeling and Surveillance, UC Davis, USA
National Centre for Foreign Animal Disease, Canada.	Department of Homeland Security, USA	Empresa Brasileira de Pesquisa Agropecuária, Brasil
Centro de Biología Molecular Severo Ochoa, España	European Animal Health and Welfare Research Collaborative Working Group	European Commission for the Control of Foot-and-Mouth Disease
Agence Française de Sécurité Sanitaire des Aliments, France	Food and Agriculture Organization of the United Nations	Federal Centre for Animal Health, All Russian Research Institute for Animal Health, Russia
Institute for Animal Health Pirbright Laboratory, United Kingdom	Merial, France	Indian Veterinary Research Institute, Bangalore, India
Centrum voor Onderzoek in Diergeneeskunde en Agrochemie, Belgium	Pfizer Animal Health, USA	National Agriculture and Food Research Organization, Japan
International Livestock Research Institute of Nairobi, Kenya	Tetracore, Inc., USA	Ohio State University Veterinary College, USA
National Veterinary Institute of the Technical University of Denmark	United States Animal Health Association	The Boyd Orr Centre for Population and Ecosystem Health, University of Glasgow, United Kingdom
Agricultural Research Council, South Africa	VALLÉE S.A, Brasil	USDA-Animal and Plant Health Inspection Service, USA
Instituto Nacional de Tecnología Agropecuaria, Argentina	Vietnam Department of Animal Health, Epidemiology Division, Vietnam	
National Veterinary Research & Quarantine Service, Korea	World Reference Laboratory for FMD, United Kingdom	
USDA- ARS, Foreign Animal Disease Research, Plum Island Laboratory, USA		
Wageningen University and Research Centre, The Netherlands		

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