Foot-and-Mouth Disease Gap Analysis

Workshop Report

December 2010

Global Foot-and-Mouth Disease
Research Alliance

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.

Additional information on the GFRA and the work of the alliance can be found on the following website: http://www.ars.usda.gov/GFRA

The purpose of the FDM Gap Analysis Workshop was to assess current scientific knowledge and the available countermeasures to effectively control and mitigate the impact of an FMD outbreak in the United States, and also support global control and eradication initiatives in FMD-endemic countries.

The FMD Gap Analysis Workshop was organized by the GFRA with the support of the United States Department of Agriculture USDA) and the Instituto Nacional de Technologia Agropecuaria (INTA).

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GLOSSARY

AGID: Agarose gel immuno-diffusion

APHIS: Animal and Plant Health Inspection Service

ARS: Agricultural Research Service

BSL: Bio Safety Level

CFT: Complement Fixation Test

DIVA: Differentiating Infected from Vaccinated Animals

EITB: Enzyme-linked immunoelectrotransfer blot assay

ELISA: Enzyme-linked immunosorbent assay

FADDL: Foreign Animal Diseases Diagnostic Laboratory, APHIS, USDA, PIADC, Orient Point, New York

FADRU: Foreign Animal Diseases Research Unit, ARS, USDA, PIADC, Orient Point, New York

GMP: good manufacturing practice

HSPD-9: Homeland Security Presidential Directive Nine

IAH: Institute for Animal Health, Pirbright, UK

Ig: Immunoglobulin

LFD: Lateral Flow Device

LPBE: Liquid Phase Blocking ELISA

MAb: Monoclonal Antibody

NAHLN: National Animal Health Laboratory Network

NSP: Non-Structural Proteins

NVS: National Veterinary Stockpile

NVSL: National Veterinary Services Laboratories

OIE: World Organisation for Animal Health

PIADC: Plum Island Animal Disease Center, Orient Point, NY

PCR: Polymerase Chain Reaction.

PPE: Personal Protective Equipment

PFU/ml: Plaque Forming Unit/milliliter

RNA: Ribonucleic Acid

RT-PCR: Reverse transcriptase-polymerase chain reaction

rRT-PCR: Real-time reverse transcription-polymerase chain reaction

SDLPBE: Single Dilution Liquid Phase Blocking ELISA

SP: Structural Proteins

SPBE: Solid Phase Blocking ELISA

SPCE: Solid Phase Competitive ELISA

VI: Virus Isolation

VNT: Virus Neutralization Test

EXECUTIVE SUMMARY

A group of international experts on Foot-and-Mouth Disease (FMD) was convened to conduct a gap analysis of our current knowledge of FMD and the available countermeasures to effectively control and mitigate the impact of an outbreak in the United States, and also support global control and eradication initiatives in FMD-endemic countries. The Foot-and-Mouth Disease Countermeasures Working Group (FMDCWG) was organized with the support of the Global Foot-and-Mouth Disease Research Alliance (GFRA) and the Instituto Nacional de Technologia Agropecuaria (INTA). The working group met in Buenos Ares, Argentina, August 24-25, 2010.

Gap Analysis

The FMDCWG identified several obstacles to effectively prevent, detect, and control FMD, including:

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

The FMDCWG determined that effective countermeasures were available but several weaknesses were identified:

Depopulation, Disinfection, and Decontamination

Depopulation is the first line of defense against an FMD outbreak in an FMD-free country when the outbreak is within a defined zone. Depopulation is the primary countermeasure to reduce virus shedding and stop the spread of the FMD virus. Disposable clothing, depopulation supplies, disinfectants, and decontamination equipment is appropriate for use in the event of an outbreak of FMD. Species-specific response packs and vaccination equipment should be stockpiled. Problems include the lack of disposal options for infected carcasses and the lack of trained, coordinated response teams to assist with rapid depopulation.

Vaccines

The group determined that the currently available inactivated vaccine antigen banks and commercially available FMD vaccines will provide an essential adjunct role in the control and eradication of FMD, especially if the virus spreads beyond the initial defined zone. These vaccines are compatible with a strategy based on "differentiating infected from vaccinated animals" (DIVA). However, there are significant differences between different manufacturers, and vaccines distributed for use in either FMD-endemic regions versus FMD-free countries. Accordingly, acquisition of any commercial vaccine for stockpiling will require an in-depth investigation and due diligence evaluation of the manufacturer and the product for sale to determine the actual profile of the vaccine for the purpose of suitability for control and eradication. Continued development of molecular FMD vaccine platforms is advised to produce improvements in the spectrum of protection against multiple serotypes, vaccine markers and companion diagnostic test for DIVA, the need for multiple doses, the onset and duration of immunity, and engineer new FMD vaccines that can be safely manufactured.

Diagnostics

Several commercial serologic (antibody-based) diagnostic test kits, including DIVA test kits have been developed in the U.S and internationally. Validated real-time (r)RT-PCR assays have been developed for use by the NVSL and NAHLN network; reagents for 96 well, robotic extraction procedures and PCR kits should be considered for possible stockpiling or contracted access. Pen-side tests could be a powerful tool in an outbreak situation if distributed to first responders/field veterinarians where test results could be obtained rapidly. Rapid antigen- and/or genetic-based pen-side tests are in development and being evaluated and validated for use. All tests should be evaluated for compliance to U.S. standards and considered for possible stockpiling or contracted access.

Recommendations

The implementation of research priorities in the following critical areas were determined to be paramount to address the gaps in our scientific knowledge and advance the availability of effective countermeasures.

Epidemiology

- 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

Viral Pathogenesis

- 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.
- Determine the early events in FMDV pathogenesis in swine and small ruminants (i.e., primary site of replication, mechanisms of spread)

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
- Support research on the immunological mechanisms of cross protection in susceptible species
- Determine the role of cellular innate immune responses in FMDV infection of cattle and swine
- Develop methods to activate cells of the innate response to anti-viral activity (NK cells, $\gamma\delta$ 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 IFNα and β as well as porcine IFNβ
- Contract the development of antibodies to surface markers of critical immune bovine and porcine cell types
- Support basic research to understand the Type I interferon locus in cattle and swine and how the protein products of these genes affect innate and adaptive immune responses
- Determine the differential expression of the IFNα genes in bovine and porcine
- Develop technologies for analyzing the adaptive immune response to infection and vaccination
- Determine correlates between cellular immune responses and vaccine efficacy

Vaccines

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

Biotherapeutics

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

Diagnostics

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

Disinfectants

- Development of low cost commercially available disinfectants for use in the inactivation of FMDV on contaminated surfaces found in farm settings and other susceptible environments.

INTRODUCTION

Foot-and-Mouth Disease (FMD) is one of the most infectious viral diseases known with devastating economic, social and environmental impacts. FMD is caused by a virus of the family Picornaviridae, genus Aphthovirus (the FMD virus [FMDV]), which has seven immunologically distinct serotypes (O, A, C, SAT1, SAT2, SAT3 and Asia 1). Additionally, a large number of subtypes have evolved within each serotype, with the end result that FMD is often considered as at least 7 distinct diseases. FMDV is transmitted by direct or indirect contact from animate and inanimate vectors, and may spread over great distances with movement of infected or contaminated animals, products, objects, and people. Airborne spread may occur up to 60 km (40 miles) overland and 300 km (190 miles) by sea, especially in temperate zones.

It is highly contagious to bovidae, suidae, cattle, sheep, goats, swine, and all wild ruminants. The morbidity of FMD is high in infected adult livestock. The disease is rarely fatal in adult animals, but often high mortality is seen in young animals due to myocarditis. Following infection, there is an incubation period of 2 to 21 days (average 3 to 8) with large amounts of virus excreted by infected animals before clinical signs are evident. Infected animals exhibit blisters and ulcers on the mouth, tongue, lips, feet and udder. Animals salivate excessively, have fever, sore feet, lose weight and stop producing milk. On recovery from FMD, approximately 50% of ruminants become 'carriers' with persistent sub-clinical infection. These animals present a critically important risk to susceptible animals as reservoirs of infection. Unfortunately, available vaccines may not protect animals from carrier status.

The disease is endemic in Africa, Asia, the Middle East and South America. Recently, there has been a notable increase in the incidence of FMD outbreaks reported in Asia and a concurrent spread of the Pan-Asia strain type O, which was the causative strain of the 2001 outbreak in the United Kingdom. FMD infection remains high throughout the world. In the last ten years, FMD epidemics have occurred in many FMD-free countries or regions, including Greece (2000) (Leforban Y. and Gerbier G., 2002), Taiwan (1997) (Yang P. C. et al, 1999), Argentina, Uruguay (2000-2001) (Correa M. E. et al, 2002), Brazil (2000, 2001, 2005) ((Correa M. E. et al, 2002; OIE, October 14, 2005), Peru (2004) (OIE, June 18, 2004), Russia (2005) (OIE, June 17, 2005), the U.K., Ireland, France, the Netherlands (2001) (Leforban Y. and Gerbier G., 2002) and the Republic of Korea and Japan in 2010.

The World Reference Laboratory for FMD, Pirbright, United Kingdom, has recently recommended the division of circulating FMD viruses into seven regional pools, based on the observation that genetically distinctive virus strains tend to occur within a defined region. The seven regional pools are 1) Eastern Asia, 2) Southern Asia, 3) Euro-Asia, 4) Eastern Africa, 5) Western Africa, 6) Southern Africa, and 7) South America. Within those pools, FMD viruses circulate and, incidentally, infect regions endemically infected by other pools or free regions of the world. For example, between January and July 2010, China, Japan, the Republic of Korea, Namibia, Mongolia, Hong Kong, Zimbabwe, Kazakhstan, Taiwan, and Russia have submitted immediate notification reports to the Office International des Epizooties (OIE, the World Organisation for Animal Health) as a consequence of FMD virus introduction into free regions or perceived changes in the epidemiological situation of the disease.

The OIE currently recognizes 65 countries without and one country with vaccination as FMD-free; 13 other countries have "regions" that have been recognized as FMD-free by the OIE. In most FMD-free regions in which virus introduction is reported, outbreaks are usually managed by the slaughter of at least some of the infected and in-contact animals. Although this restores FMD-free status, such widely publicized culls of livestock are increasingly controversial due to loss of genetically optimized breeding stock, and community, economic and environmental concerns.

The impact on FMD-free countries is evidenced by the introduction of FMD to the United Kingdom in 2001, which resulted in millions of livestock being slaughtered and economic losses conservatively estimated to be U.S $14.7 billion (Anderson 2001). The disease's repercussions were felt broadly: beyond the agricultural sector and supporting rural communities, work force mobility and tourism were both significantly affected by the outbreak. The potential of the disease to disrupt normal social and economic function underscores the need for high levels of expenditure on surveillance and emergency preparedness.

The arsenal of FMD management tools currently available to farmers, veterinarians and governments is inadequate to manage FMD. Control of the spread of the disease is predominantly by physical interventions. Vaccines have limited utility in an acute outbreak as they are slow to offer immunity and are relatively short acting. Even after vaccination, animals can become FMDV carriers and there are currently limitations with our ability to reliably distinguish infected from vaccinated animals. Further, there are no licensed therapeutic options available and so there is urgent need to improve the range of products available to manage FMD.

Disease experts have consistently rated FMD as the most significant threat to the U.S livestock industries (see Expert Reports on Page 19). Accordingly, an FMD Countermeasures Working Group (FMDCWG) was charged with the task of conducting an in-depth analysis of available countermeasures to control and eradicate FMD should an outbreak ever occur in the U.S. This report provides the results of this analysis. The FMDCWG used a decision model to objectively compare available countermeasures, focusing primarily on vaccines and diagnostics. Because current commercial products were not specifically designed for the control and eradication of FMD, the FMDCWG also assessed experimental vaccines and diagnostics considered to be in the "pipeline" and reachable. Other countermeasures such as biotherapeutics, disinfectants, and personal protective equipment (PPE) were also assessed.

BACKGROUND

Organization of the Foot-and-Mouth Disease Countermeasures Working Group (FMDCWG)

An international team of FMD experts from research institutions, industry, academia, and government was selected by the Chair to serve on the FMDCWG. The FMDCWG workshop was organized with the support of the Global Foot-and-Mouth Disease Research Alliance (GFRA) and the Instituto Nacional de Technologia Agropecuaria (INTA). A total of 15 experts (see list of working group members on pages 3-6) accepted to serve on the FMDCWG. The FMDCWG met in Buenos Ares, Argentina, August 24-25, 2010. Instructions (see Appendix I) and several reference materials were provided by the FMDCWG Chair prior to the meeting. The FMDCWG members were tasked by the Chair with assessing the best available countermeasures to rapidly and effectively control and eradicate FMD should an outbreak occur in the United States. When gaps in the information necessary to complete the analysis were identified, FMDCWG members contacted additional experts directly (see list of ad hoc contributors on page 7).

Expert Reports

The FMDCWG used the following reports as background information on the risks of a FMD introduction occurring in the United States.

The USDA Foot-and-Mouth Disease Response Plan – The Red Book

The USDA National Animal Health Emergency Management System (NAHEMS) Guidelines: Vaccination for Contagious Diseases, Appendix A: Foot-and-Mouth Disease

2006 Foot and Mouth Disease Summit Report. Hosted by the Animal Health Network and National Cattlemen's Beef Association. http://www.fmdinfo.org/uDocs/pdfofbookforweb767.pdf

Strategic research targets to protect American livestock and poultry from biological threat agents. Report from the WMD Counter Measures Working Group -Animal Pathogen Research and Development Subgroup. http://www.ars.usda.gov/research/programs/programs.htm?np_code=103&docid=5815

The Office of Science and Technology Policy (OSTP) Blue Ribbon Panel on the Threat of Biological Terrorism Directed Against Livestock. Conference Proceedings, Washington DC, December 8-9, 2003. http://www.ostp.gov/html/STPI.pdf

Centers for Epidemiology and Animal Health (CEAH)-VS-APHIS-USDA Report (April 2006). Foot-and-Mouth Disease: Sources of Outbreaks and Hazard Categorization of Modes of Virus Transmission. USDA/APHIS/VS/CEAH/CADIA, Natural Resources Research Center, Bldg. B, 2150 Centre Avenue, Mailstop 2W4, Fort Collins, CO 80526-8117.

European Food Safety Authority (EFSA) Journal (2006) 313, 1-34. Opinion of the Animal Health and Welfare Panel related to: Assessing the risk of Foot and Mouth Disease introduction into the EU from

developing countries; Assessing the reduction of this risk through interventions in developing countries / regions aiming at controlling / eradicating the disease; Tools for the control of a Foot and Mouth Disease outbreak: update on diagnostics and vaccines. Available at: http://www.efsa.europa.eu/en/science/ahaw/ahaw_opinions/1357.html.

Food and Agriculture Organization, Emergency Prevention System (EMPRES) for Transboundary Animal and Plant Pests and Diseases, Foot-and-Mouth Situation Worldwide. Available at: http://www.fao.org/docs/eims/upload//225050/Focus_ON_1_07_en.pdf

Office International des Epizooties reports and current FMD situation. http://www.oie.int/eng/Status/FMD/en_fmd_free.htm

World Reference Laboratory for FMD, Pirbright, UK, annual and quarterly reports. http://www.wrlfmd.org/ref_labs/fmd_ref_lab_reports.htm

DEFINITION OF THE THREAT

An accidental or intentional outbreak with Foot-and-Mouth Disease Virus (FMDV) is recognized as the most significant foreign animal disease threat to FMDV-free countries and its potential as an agent of bioterrorism is widely recognized (Anonymous 1982; Borio, Inglesby et al. 2002; Sidwell and Smee 2003). The following section summarizes the status of our understanding of viral pathogenesis and epidemiology, the available tools to effectively control and eradicate FMDV, a summary of the FMD situation worldwide, and current obstacles for controlling FMD should an outbreak ever occur in the United States.

Virology

FMD is widely recognized as the most threatening agricultural disease due to its virulence, infectivity, potential impact on economic trade, and its presence and re-emergence in various parts of the world. FMD is a systemic disease of domestic and wild cloven-hoofed animals caused by FMD virus (FMDV), the prototype member of the genus *Aphthovirus* of the family *Picornaviridae.* The highly contagious nature of FMD and the associated productivity losses makes it a primary animal health concern worldwide. Seven distinct FMDV serotypes (A, O, C, Asia1, and South African Territories [SAT] SAT1, SAT2 and SAT3) and multiple subtypes reflect the significant genetic and antigenic variability of the virus. Recent comparative analysis of FMDV genomes representing the seven serotypes have shown that 42% of amino acids present in viral proteins are susceptible to change (Carrillo, Tulman et al., 2005). In addition, evidence exist for extensive intertypic recombination within the regions coding for the non-structural proteins between FMDVs sharing the same geographical location (Jackson et al 2007).

The 30 nm non-enveloped FMDV particle is surrounded by an icosahedral capsid made up of 60 copies each of four structural proteins. The capsid surrounds an approximately 8.4 kilobase, positive sense, single stranded RNA genome that functions like mRNA, that is covalently linked to a protein called VPg at the 5’ end and is flanked by highly structured 5' and 3' untranslated regions (UTR) with roles in viral translation and genome replication. The three dimensional structure of a number of FMDV serotypes has been determined by X-ray crystallography (Acharya et al. 1989). Upon virus entry into a cell, via interaction with specific receptors, the single viral open reading frame (ORF) is rapidly translated into a polyprotein, which is cleaved by viral proteinases into 14 mature proteins (Grubman & Baxt, 2004;Abrams, King et al., 1995;Clarke & Sangar, 1988;Grubman & Baxt, 1982). The four capsid proteins, 1A, 1B, 1C, and 1D (also known as VP4, VP2, VP3, and VP1, respectively) are encoded within half of the ORF and, with the exception of 1A, are involved in immunogenicity and binding to cell receptors. Non-structural proteins include L^{pro}, 2A, 2B, 2C, 3A, $3B_{1-3}$, $3C^{pro}$, and 3Dpol. L^{pro}, $3C^{pro}$, and 2A are proteases that mediate cleavage of the viral polyprotein and, in the case of L^{pro} and $3C^{pro}$, they also cleave specific host proteins (Belsham, McInerney et al., 2000;Birtley & Curry, 2005;Burroughs, Sangar et al., 1984;Falk, Grigera et al., 1990;Gradi, Foeger et al., 2004). The functions of 2B, 2C, and 3A are unclear but they have been implicated in host tropism and in association with membranes. Protein 3B (Vpg) is linked to the 5’ end of the genome and is required for viral RNA replication and 3D encodes the viral RNA-dependent RNA polymerase (Mason, Grubman et al., 2003) (see Figure 1 for details).

FMDV infectious cycle

The infectious cycle begins with the interaction of FMDV VP1 with the cell surface (attachment) through specific receptors, (integrins αvß1, αvß3, αvß6 and αvß8) leading to virus docking onto target cells [reviewed by Baxt et. al (Baxt & Rieder, 2004; Baxt, Neff et al., 2002)]. Alternative receptors, most significantly heparin sulfate and a third unknown receptor, can mediate FMDV infection *in vitro* (Jackson, Ellard et al., 1996)(Baranowski et al 1998, Zhao et al 2003). After adsorption and penetration the virus disassembles in an acidic endosomal compartment releasing the RNA into the cytosol. The viral RNA is rapidly translated into the polyprotein by a cap-independent mechanism controlled by the 5'UTR whereas cap-dependent host translation is suppressed by L^{pro} via proteolytic cleavage of host elongation factor 4 (Li, Ross-Smith et al., 2001;Belsham, McInerney et al., 2000). This mechanism of suppression of cellular protein translation is one of the few well characterized FMDV genetic determinants of virulence (de Los, de Avila et al., 2006; Devaney, Vakharia et al., 1988). Cleavage of the polyprotein by viral proteases results in accumulation of structural and non-structural proteins in the cytoplasm. Synthesis of minus and plus-strand RNA by viral replication complexes takes place in endoplasmic reticulum-derived membranes by poorly understood mechanisms, and is followed by encapsidation of plus-strand RNA and virion maturation. Release of progeny particles occurs as soon as 4 to 6 hours post infection (hpi). Virus interference with cell processes leads to biochemical and morphological alterations that result in cell death under cytolytic infection conditions.

Animal infection

FMDV causes an acute disease characterized by fever, lameness, and vesicular lesions on the feet, oral cavity, snout, teats, and other epithelial sites. These debilitating effects, rather than high mortality rates, are responsible for the severe productivity losses associated with FMD. FMDV spreads by direct or indirect contact with infected animals or their secretions. Infection of cattle generally occurs via the respiratory route by aerosolized virus (Donaldson, Gibson et al., 1987, Arzt et al., 2010) while pigs usually become infected by eating virus-contaminated food or through skin lesions while in contact with infected animals (Alexandersen, Quan et al., 2003). Pigs require a larger amount (10-100 fold) of virus than cattle for aerosol infection (Alexandersen & Donaldson, 2002). Replication at primary sites is followed by viremia that usually coincides with high fever (up to 41° C). FMDV can persist in domestic and wild ruminants with viral shedding in the oral-pharyngeal fluid for long periods of time (Sutmoller & Gaggero, 1965;Burrows, 1966; McVicar & Sutmoller, 1969; Hedger & Condy, 1985; Moonen & Schrijver, 2000). However, the true extent of the threat of contagion to naïve animals from persistently infected ruminants remains poorly defined (Golde, Pacheco, et al. 2005).

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Pathogenesis

Though many aspects of the pathogenesis of FMD remain incompletely elucidated, it is clear that rapid systemic dissemination with high titer viral replication and dysregulated host immune responses are central to the observed pathological processes. Major gaps in our understanding of the molecular events of early pathogenesis limit the design and development of completely effective countermeasures. Yet, it is becoming increasingly apparent that the early stages of disease are characterized by pan-respiratory tract infection. Thus, enhancement of mucosal immunity is likely to produce a substantially improved prophylactic effect.

It is well-established that the respiratory tract is the most important route of infection of FMDV in cattle (Burrows et al., 1981 Oct; Brown et al., 1996). For many years, conflicting data from different research

groups have implicated regions of either upper respiratory tract (nasopharynx) or lower respiratory tract (lungs) as the primary sites of infection. Recent work has demonstrated that after aerosol exposure to the virus, ”the temporally defined early pathogenesis events involve (1) primary replication in epithelial cells of the pharyngeal mucosa-associated lymphoid tissue crypts and (2) subsequent widespread replication in pneumocytes in the lungs, which coincides with (3) the establishment of sustained viremia” (Arzt, et al, 2010.). This model demonstrated that massive viral amplification occurs in the lungs (with associated shedding to the environment) prior to appearance of the first vesicle. Viremia is established coincidently with further viral amplification in the lungs and at lesion (vesicle) predilection sites. In the context of countermeasures development, it should be noted that it is critical that prophylactic products target these previremic events in the pharynx and lungs. Thus, enhancement of mucosal immunity has high probability of improving protection. Once viremia is established, on an individual animal basis, the battle has already been lost. Additionally, continued efforts to improve the understanding of virus host interactions during early phases of infection will greatly contribute to the development of effective tools to block viral infection.

The clinical phase of disease is characterized by fever and rapid dissemination of FMDV to secondary sites of infection, most significantly in the skin and other stratified squamous epithelia, where virus is greatly amplified; the classic vesicular lesions develop only at specific and consistent sites of friction (coronary bands, oral cavity, snout, tongue, prepuce and teat skin) despite widespread virus dissemination (Alexandersen, Zhang et al., 2003; Hess, 1967; Burrows, Mann et al., 1981; Arzt et al., 2009).

Clearance of virus from blood occurs 2 to 3 days after viremia is first detected, followed with the appearance of circulating antibodies (Cottral & Bachrach, 1968; Cottral & Bachrach, 1968). Elimination of virus at secondary sites of infection usually takes 10 to 14 days (Oliver, Donaldson et al., 1988; Oliver, Donaldson et al., 1988). In domestic and wild ruminants, FMDV may persist (i.e. carrier state) with intermittent viral shedding in the oral-pharyngeal fluid for extended periods of time (Sutmoller & Gaggero, 1965; Burrows, 1966; McVicar & Sutmoller, 1969; Hedger & Condy, 1985; Moonen & Schrijver, 2000). Persistence may result from symptomatic or asymptomatic infection of naïve, convalescent or vaccinated animals. Recent evidence suggests that the sites of viral persistence are in the pharyngeal region, specifically the dorsal soft palate, dorsal pharyngeal area, and associated lymph nodes (Zhang & Kitching, 2001; Juleff et al., 2008). The mechanisms mediating the establishment and maintenance of persistent infections in ruminants remain unclear, but it is noteworthy that both primary and persistent infections with FMDV have been associated with pharyngeal tissue in ruminants (Alexandersen, Zhang et al., 2002;Alexandersen, Zhang et al., 2003). The role of persistence in the transmission of FMDV is poorly understood, although some evidence indicates that persistently infected African buffaloes (*Syncerus caffer*) can serve as a source of infection to cattle. Despite the uncertainty surrounding the true threat posed by FMDV carriers, it is clear that the perception of threat from these animals is one of the main driving forces dictating FMD-associated trade issues. Thus one of the long term goals of novel FMD countermeasures must be prevention or cure of the carrier state.

Immunology

Immune responses to infection

FMD viruses have become successful pathogens in large part by overcoming both the host innate and adaptive immune responses allowing them to multiply sufficiently to be transmitted to new hosts or establish viral persistence. Most of the published work on the immune mechanisms elicited after infection has been conducted in swine and laboratory animal models (mainly mice). However, there are profound differences in the immune responses and protection mechanisms between cattle and swine, as well as mice. Therefore, not all the results obtained for these species may be necessarily valid for bovines or small ruminants.

These observations are linked to differences in FMDV pathogenesis among animal species. As it was mentioned before, ruminants, in contrast to pigs, are highly susceptible to infection by the respiratory route. They may be infected experimentally by airborne exposure with doses over 10^3 times lower than pigs (reviewed in (Alexandersen, Zhang et al. 2003). This coincides with differences in the primary sites of viral replication and primary responses to infection between these two animal species. Regarding the mouse model, replication of FMDV has been reported to occur mainly in the pancreas (Fernandez, Borca et al. 1986). Consequently, mice have traditionally been used mainly for studies on systemic immunity, but not for mucosal/local immunity. Moreover, the disease in mice is highly variable and dependant on the mouse strain; while the disease is subclinical for adults from most strains, for others (C57/Black) FMDV have been shown to be acute and lethal (Salguero, Sanchez-Martin et al. 2005).

Lymphopenia and immunosuppression have been reported to occur early in pigs post-infection (Diaz-San Segundo, Salguero et al. 2006; Diaz-San Segundo, Rodriguez-Calvo et al. 2009), though these observations have been seldom recorded in cattle. Also, the induction of protective immunity against viral challenge in the absence of antibodies has been reported to occur very early post-immunization with experimental FMDV vaccine formulations in swine (Moraes, de Los Santos et al. 2007) and mice (Molinari, Garcia-Nunez et al. 2010). These findings have not been reported for cattle.

Experimental studies evaluating persistent infections have demonstrated that only some ruminant species exposed to FMDV become carriers, irrespective of whether they are fully susceptible or immune; i.e., protected from disease as a result of vaccination or recovery from infection. These observations are associated with differential patterns of humoral and mucosal immune responses in these animals (Moonen, Jacobs et al. 2004; Maddur, Gajendragad et al. 2008) with a high level of systemic antibodies observed for at least seven months after infection (McVicar and Sutmoller 1974), and a persistent presence of low levels of IgA in esophageal-pharyngeal fluids (Parida, Anderson et al. 2006).

In vitro studies have demonstrated that FMDV is highly sensitive to type I interferons (IFN) (Chinsangaram, Piccone et al. 1999). Multiple dendritic cell (DC) subsets release IFNα when exposed to FMDV (Bautista, E. et al., 2005; Nfon, Ferman, et al, 2008) or FMDV/antibody immune complexes (Guzylack-Piriou L., et al, 2006). Like other viruses, FMDV has evolved a variety of strategies to circumvent this response. FMDV expression of leader protease (L^{pro}), functions as an antagonist to host cell protein synthesis including IFNα, IFNβ and IFNγ (Grubman M. J., et al, 2004). Moreover, viral protein 2B in conjunction with 2C or their precursor 2BC inhibits protein trafficking through the endoplasmic reticulum and Golgi apparatus. A decrease in surface expression of major

histocompatibility class I molecules during FMDV infection suggests that 2B, 2C and/or 2BC may be involved in delaying the initiation of the host adaptive immune response and also adversely affect the secretion of induced signaling molecules (Grubman, Moraes et al. 2008).

In vivo studies carried out in swine also showed that the virus must overcome the very rapid IFNα/β response in order to establish a successful infection (Chinsangaram, Mason et al. 1998). However, during infection in pigs, the IFNα response of multiple dendritic cell subsets is inhibited (Nfon, Dawson et al. 2008). Studies examining the early immune response of swine to FMDV revealed a viral induced, transient lymphopenia in the circulation during the acute phase of infection and yet lymphocytes are not infected and therefore not killed by the virus (Bautista, Ferman et al. 2003). Later reports, however, have shown that FMDV can infect some swine T cells subsets following a lytic cycle (Diaz-San Segundo, Salguero et al. 2006). Further, the IFNγ response of T cells and NK cells is depressed and often completely blocked during lymphopenia (Bautista, Ferman et al. 2003; Toka, Nfon et al. 2009).

Infected cattle are fully protected against further infection of the homologous strain by antibody-mediated mechanisms. Antibody secreting cells can be detected all along the respiratory tract as soon as 4 days post infection (dpi), leaving a narrow window with the onset of the systemic responses, assessable between 4 and 5 dpi (Pega, Bucafusco et al. 2010). Viremia is no longer detectable by qRT-PCR or viral isolation from 6 to 10 dpi (Arzt, Pacheco et al. 2010), depending on the strain.

IgA antibodies are induced shortly after infection and maintained with low titers in carrier animals for at least 210 days post-infection (Salt, Mulcahy et al. 1996). IgG1 isotype has been associated with protection in vaccinated cattle. Significant levels of serum FMDV-specific IgG1 over IgG2 have been measured at 14 dpi in infected animals (Capozzo, Periolo et al. 1997). McGuire et *al.* (McGuire, Musoke et al. 1979) found that both bovine IgGl and IgG2 antibody-antigen complexes are able to fix bovine complement *in vitro* but IgGl might be more efficient than IgG2. This, together with its better capacity to interact with FcR on phagocytes, could further promote the opsonization-enhanced phagocytosis by cells of the reticuloendothelial system. However, the role of the different isotypes in clearance of the viremia at earlier times post infection (within one week of the infection) still needs to be established.

In contrast to the well-defined role of humoral immune responses, the contribution of T-cell-mediated responses to immunity and their role in the induction of protective B-cell responses to FMDV in the natural host species are poorly understood.

Very early reports by Borca et al. showed that the protective immune response against FMDV in a murine experimental model was T cell independent (Borca, Fernandez et al. 1986). Recent work suggests that functional CD4+ T cells are not required for controlling FMDV primary infection in cattle. Isotype switching of the antibody response was also found to be independent of CD4+ T cells (Juleff, Windsor et al. 2009).

CD8+ T-cell responses to FMDV in livestock had been proposed only for infected animals, but the T-cell proliferation assays employed were unable to demonstrate whether or not the detected responses were class I major histocompatibility complex (MHC) restricted (Childerstone, Cedillo-Baron et al. 1999). In the same way, cytotoxicity assays were only successful in antigen presenting cells (APC) loaded with FMDV peptides but not in actual FMDV-infected cells (Guzman, Taylor et al. 2010). As a

whole, a physiological role for a T-cell cytotoxicity mechanism in protection to FMDV in cattle has yet to be clearly demonstrated.

There are only a few reports describing expression of pro-inflammatory cytokines in different target tissues in cattle (Zhang, Bashiruddin et al. 2006; Zhang, Ahmed et al. 2009), though no conclusive evidence of mechanisms similar to those described in pigs have been reported.

It is worth noting that although FMDV infection can be fatal to young calves, immunological as well as pathogenesis studies have been mostly conducted in adult animals. There is no comprehensive information on immune responses of calves to FMDV infection, kinetics of viral clearance or influence of maternal immunity.

Immune responses to vaccines

Immune responses to vaccines have been mostly assessed in cattle, probably due to its higher relative economic impact compared to other livestock species. Presently, vaccination against FMDV is done with a chemically killed virus preparation in oil or aqueous-saponine adjuvants (Doel T. R., 2003). As previously mentioned for viral infection, protection provided by FMDV-vaccines is strain-specific, so all regionally-circulating strains should be included in the formulation. Vaccination prevents clinical disease but not viral infection, nor the eventual viral persistence (carrier state). However, as previously stated, although the carrier state has been documented and studied in naïve and vaccinated cattle (Alexandersen, Zhang et al. 2002; Kitching 2002), transmission of FMD has never been convincingly demonstrated under controlled conditions (Sutmoller, Barteling et al. 2003).

Vaccine-induced protection is mediated by an antibody response and can be predicted by means of Virus Neutralization Test (VNT) and Liquid Phase blocking ELISA (LP ELISA) performed at 60 dpv with strain-specific monoclonal antibodies (Robiolo, Grigera et al. 1995; Robiolo, La Torre et al. 2010). Measurement of IgG1 levels have been related with protection, even in vaccines inducing low LP ELISA titers (Capozzo, Periolo et al. 1997) suggesting that antibody quality should be assessed as well.

FMDV-vaccines induce seroconversion shortly after administration. Immunity induced by a commercial monovalent O1 Manisa vaccine with a regular payload (3PD50) conferred complete protection at 7 days post vaccination (dpv; Golde, Pacheco et al., 2005). Interestingly, in that same study, partial protection, including no fever, no viremia, and delayed disease were observed when the cattle were challenged 4 days after vaccination.

Vaccine immunity is T-cell dependent and these responses are heterotypic (Collen and Doel 1990). T-CD4+ cells from vaccinated animals produce IFN-γ in that can be easily measured *ex vivo* by cultivating whole blood with viral antigens (Parida, Oh et al. 2006; Bucafusco, Pega et al. 2010).

Efficacy evaluation of experimental recombinant vaccines highlighted that protection can be achieved by other mechanisms than antibodies. In this regard, a human adenovirus 5 viral vectored vaccine has shown complete protection at 21 days following vaccination (Mayr G.A., et al, 1999; Moraes M. P., et al, 2002). However, the vaccine also protects against disease as early as 7 dpv (Moraes M. P., et al, 2002; Pacheco J. M., et al, 2005), with minimal or absent antibody response. One hypothesis to explain

this protection suggests induction of an innate response including IFN release from DCs and other cells and/or activation of NK cells and other innate response cells.

Analyses of innate and cellular responses have been hindered by a lack of reagents and experimental capacity to clearly define the parameters of the response(s) that confer protection. These deficits include a lack of antibodies specific for bovine IFNα and β as well as porcine IFNβ, a lack of understanding of the differential expression of the many IFNα genes in these species, and no antibodies specific for surface markers identifying critical immune cell types.

One of the important gaps with FMD vaccination is the lack of cross-protection across serotypes, and subtypes, especially in highly variable FMDV Serotype A and SAT strains. For serotype A, vaccines formulated with high antigen payloads have showed improved ability to cross-protect (Brehm et al. 2008); although this was not described for other serotypes. Gaps in this area also include the role of cross-reactive FMDV cellular immune responses (CD4+ and CD8+) in protection and the improved capacity of multivalent vaccines to broaden heterotypic protection against other strains not included in these formulations. A hypothesis to explain this phenomenon is that the immune system appears to be decoyed into reacting to immunodominant epitopes that offer little cross-protection between serotypes or subtypes. Because the propensity of the immune system to react against immunodominant strain-specific epitopes appears to be genetically hard-wired, this phenomenon has been termed "deceptive imprinting." An important area of research related to deceptive imprinting are strategies for overcoming this phenomenon in the design of vaccines (Tobin et al., 2008).

Very few reports have been focused on neonatal immunity to FMDV vaccines. Interference of maternally-transferred antibodies does not seem to completely abrogate the induction of antibody responses by vaccination (Späth, E.J.A., et al., 1995). Immune cells transferred via colostrum might modulate neonatal immune responses (Donovan, Reber et al. 2007); however, this issue has not been explored so far for FMD.

The capacity of young animals to mount a protective response to vaccination in the absence of maternal immunity has only been addressed by two very early reports (Nicholls, Black et al. 1984; Sadir, Schudel et al. 1988). In these studies, oil and hydroxide-saponine adjuvanted vaccines proved to be inefficient when applied to 1-7 days old animals, though they may elicit seroconversion when administered after 21 days of life. Further studies are needed to determine the intrinsic ability of the neonatal immune system to respond to commercial vaccines and to determine the time-span between vaccination and protection.

In summary, the actual efficacy of commercial FMD vaccines under "real world" field conditions is variable, in large part due to differences in vaccine formulations (e.g., adjuvant, payload, and potency), variations in the immune response in different hosts (cattle, swine, and sheep), genetic background and animal age. Further development of FMD countermeasures requires advances in our knowledge of the innate and adaptive response of cattle and swine to FMD viral infection.

Epidemiology

FMD is considered to be one of the most contagious infectious animal diseases in the world and typically inflicts severe and far-reaching economic losses throughout infected countries (James A. D. and Rushton, 2002). Since the first description of the disease nearly five centuries ago (Fracastorii H, 1554), the FMDV has been found in more than 70 species, including cattle, buffalo, sheep, goats, pigs,

and deer (Grubman M. J. and Baxt B., 2004). FMD viruses are genetically very diverse, with seven immunologically distinct serotypes (A, O, C, SAT1, SAT2, SAT3, and Asia1). The disease is usually characterized by high morbidity and low mortality, except in young animals in which death from cardiac involvement is common (Kitching R. P. and Hughes G. J, 2002). Clinical signs of the disease are depression, lameness, and appearance of vesicles progressing to epithelial erosions and ulcers in the oral cavity, on the teats, and/or in the interdigital space. Some strains of the virus and some host species show minimal or no signs of disease. FMDV transmission occurs by direct contact between an infected animal and susceptible animals, or by indirect contact between susceptible animals and contaminated people, vehicles, or other contaminated items on which the virus may remain viable for up to15 weeks (Cottral G. E., 1969).

Reporting of FMD outbreaks, factors related with FMD transmission and spread, and identification and sequencing of FMD virus strains is based on the voluntary submission of information, reports and clinical samples to international organizations, rather than to the active collection of information and application of targeted sampling schemes. There is no global surveillance system for real-time reporting, visualization, analysis, and long distance communication of spatial and temporal distribution and incidence of FMD. Moreover, the informatics technology and analytical tools required for the development and support of a global surveillance system are still at the initial steps of research and development.

To compensate for lack of recent experience with the FMD outbreaks in the U.S, models for FMD spread have been developed to simulate the expected spread of the disease in the U.S. and to identify the most cost-effective combination of control strategies. However, these models are not intended to be used in helping the decision making process in the face of an epidemic, but to provide more general estimates of how an FMD epidemic would behave under certain conditions or assumptions. The consequences of misusing simulation models were dramatically demonstrated during the FMD epidemic that affected the U.K. in 2001. Attributes of a new generation of simulation (‘intelligent’) models must include the ability to capture information emerging from the field in the face of an epidemic, to use that information to adapt the model parameters (‘learning’), to modify model assumptions, including those related with the characteristic of the strain causing the outbreak, and to produce updates in near-real time that correct previous estimates of the expected evolution of the epidemic.

The emergence of new variants of FMDV is common. In the past, some of the atypical strains have spread to regions of the world far away from the originating country. The best documented case is the spread of the serotype O Pan-Asia strain. Changes in the virus genome could affect pathogenesis to the extent that typical animal species are not infected, such as the porcine-adapted strain in Taiwan or a particular vaccine strain may have diminishing protective ability against new strains. The role of carrier state in the evolution of strains has been proposed, but the impact in the field is still unclear. We do not know, for example, if shedding can be triggered by other infectious diseases, such as Bovine Viral Diarrhea (BVD). We know very little about the epidemiology of viral adaptation to host species, vaccination status, environment (physical forces of heat, desiccation or how does disease manifestation (e.g., morbidity, mortality, duration, etc) affect virus evolution. In summary, little is known about the nature and extent in which ecological and epidemiological factors and forces influence the persistence, emergence, competition, and spread of new variants of FMD viruses.

Summary of FMDV Field Strain Characteristics

- FMDV Serotype O is the most prevalent worldwide
- FMDV Serotype A is the second most prevalent
- FMDV Serotype O is the most prevalent serotype in South America
- FMDV Serotype Asia 1 is detected primarily in the Indian subcontinent and is thought to be associated with the Asian water buffalo (*Bubalus bubalis*)
- FMDV Serotypes A and SAT 2 are more hypervariable than other serotypes
- FMDV Serotypes C and SAT 3 are the least prevalent, with C possibly extinct

Diagnosis

An effective response to an FMD outbreak requires a comprehensive diagnostic plan that addresses each phase of the outbreak (surveillance, response and recovery). Early detection and surveillance are the keys to controlling the spread of the virus and reducing the economic impact of an outbreak. During the initial stages of response, surveillance efforts in the areas surrounding the quarantine zone will require testing large numbers of samples. Tests that are needed during this period include laboratory based antigen and nucleic acid detection assays that are rapid, sensitive, highly specific and adaptable to high throughput. During the recovery phase of an outbreak, serological assays that are capable of differentiating vaccinated versus infected animals should be utilized. These assays should also be rapid, high-throughput and adaptable to automation.

Although a robust diagnostic system for detecting FMDV exists in the U.S, there are still significant gaps in our diagnostic capability:

- Diagnostic test kits that can be used during each phase of the outbreak
- Tests to rapidly detect cases in the field
- On-farm screening test for detection of FMDV in dairy holdings to allow movement of milk
- Pen-side tests that can be strategically distributed to trained veterinarians in the field and that includes a central reporting system
- Pen-side tests or mobile screening assays for rapid detection and surveillance in the surrounding quarantine zones
- Robust laboratory and field tests to determine infection in vaccinated animals (DIVA)
- Reagents for assays that are pre-determined to be “fit for purpose” and validated
- There is a need to increase the testing capability of the National Animal Health Laboratory Network (NAHLN) with high throughput semi-automated robotic systems that are readily deployable

Vaccination

Conventional inactivated (killed virus) vaccines have been developed and have proven effective in reducing clinical disease in FMD-endemic areas. Recently, these vaccines have been successfully used as an adjunct treatment in disease eradication programs in Africa, Asia, South America and Europe. Despite their success as aids in prevention, control and eradication programs, shortcomings of current FMD vaccines include:

- Required adaptation of wild type virus to cell culture, usually Baby Hamster Kidney cells (BHK), for vaccine seeds can sometimes be difficult, time consuming and costly
- Virus yield can sometimes be low for some hard-to-adapt viruses

- This can be overcome by culturing virus in primary epithelial cells from cattle tongue explants (Frenkel method). These vaccines can protect with lower payloads (potency) than conventional vaccines, all of which are produced on BHK cells. A few companies still produce vaccines by the Frenkel method in South America and Europe. However, this production method is difficult to carry out following good manufacturing practices and quality-standards requirements
- Production of large volumes of wild type virus for vaccine manufacture requires high containment BSL-3 facilities and by law, FMDV cannot be produced in the U.S.
- A key concern for both FMD-free and FMD-endemic countries is the potential for FMDV escaping from manufacturing facilities
- Reduced stability of 140S particles on antigen preparation for selected FMDV strains (e.g., SAT viruses)
- Short shelf life requires banking of non-formulated antigen concentrates (One solution might be an insurance reposition contract, by which a vaccine producer keeps a stock of each vaccine lot until expiration date and then replenishes the stock so that finished vaccines are always available)
- If an outbreak were to be diagnosed in the U.S, appropriate bulk antigen(s) stored in vaccine banks would have to be identified for the strain responsible for the outbreak (vaccine matching), and appropriate concentrated antigen would have to be formulated, resulting in a 1-2 week delay
- Onset of protection takes 7-14 days
- If highly purified vaccines are not used, it is difficult to determine infection in vaccinated animals due to presence of non-structural proteins in vaccines.
- FMDV has a range of diverse serotypes and a large number of strains within some of the serotypes to which there is limited cross-immunity. There is a probability that the antigens available in commercial vaccines or antigen bank may not match or provide immunity against a new FMDV strain appearing in the field (see Figure 2).
- Many vaccine formulations fail to induce long lasting protective immune responses and require a booster dose and revaccination every six months
- Complete reliance on maintenance of cold chain through formulation to final delivery

Summary of FMD Vaccine Strain Characteristics

- FMDV Serotype O is less immunogenic
- Vaccines for FMDV Serotype O need a higher payload than Serotypes A, C, or Asia
- FMDV Serotype SAT antigens are less stable
- FMDV Serotypes A and SAT 2 are more antigenically variable than other serotypes

Economic Loss

The eradication of the 2001 FMDV outbreak in the United Kingdom is estimated to have cost U.S $14.5 billion (Anderson, 2001). While the United States is currently FMD-free, the disease poses a significant threat to the sustainability of U.S. animal agriculture. Estimates from several studies indicate far-reaching economic consequences if the U.S. acquires FMD. Direct and indirect costs estimated from a study in 1979 (McCauley E. H., et al, 1979) indicate that FMD would cost more than $37 billion over a 15-year period with values projected in year 2006 dollars. An FMD epidemic in southern California would directly cost an estimated $4.3-$13.5 billion (1999 dollar value) (Ekboir J, 1999). These estimates do not address extensive losses expected by allied livestock industries (e.g. feed, equipment,

product development), or indirectly related industries, such as was experienced by the loss of tourism and horse racing in the 2001 U.K epidemic. Other impacts would include reduced availability of animal products throughout various segments of the economy, including bovine fetal serum used in tissue culture and vaccine production and gelatin used in pet foods, nutritional supplements, and cosmetics.

FMD Situation Worldwide

In 2009, FMD remained confined to traditionally infected areas and no outbreaks were reported in countries listed by the OIE as FMD-free without vaccination (2009 annual report of the World Reference Laboratory for FMD). However, in 2010, a number of FMD-free areas have been infected by the virus, most notably South Korea, Russia and Japan. Most of the FMD viruses isolated in Pirbright were obtained from samples submitted from Africa and Asia, which remain the major reservoirs for FMDV. In South America, FMDV circulation has been reported in Ecuador and Venezuela. FMD viruses continue to circulate in vast regions of the world, including the Indian sub-continent, China, Central Asia, the Middle East, and Africa. The extensive FMD incident in Japan has dramatically reminded FMD-free countries about the continue threat imposed by the disease.

Obstacles to Prevention and Control

There are several obstacles to effectively prevent and control FMDV.

1. Poor and inadequate education and training of veterinarians and livestock producers in detecting early signs of FMD.
2. Lack of validated commercial pen-side test kits for disease control (Portable or field-based tests).
3. Failure of serologic methods to determine status (infected, uninfected) in some vaccinated animals.
4. Absence of a surveillance system for early recognition of signs, or to find evidence using antigen detection, antibody, or virus detection.
5. Lack of reliable comprehensive international surveillance systems to collect and analyze information; e.g., data on animal and animal products movement, FMD incidence and risk, and molecular epidemiology surveillance to provide estimates of international situation awareness in near-real time.
6. Current epidemiological models do not provide answers to certain questions that will emerge in the face of an FMD epidemic. Current models have not been designed to evaluate in real-time the cost-effectiveness of alternative control, surveillance, and sampling strategies, so that the results of the evaluation can be used to implement specific measures in the face of the introduction of specific FMD virus strains into the U.S.
7. Several aspects of FMD epidemiology and transmission still have to be uncovered, including the influence of viral factors that affect viral persistence, emergence, competition, transmission, and spread of FMD virus strains.
8. While several commercial vaccines are available internationally, their efficacy and safety profiles need evaluation.
9. At present, there is no rapid pen-side or field-based diagnostic test for FMD control during a disease outbreak that has been validated in the field as “fit for purpose.”
10. There is a need for analytical tools to support the decision making process in endemic settings, including, a) anomaly detection methods to identify outlier events using rule-based and model-based algorithms; b) prediction models for identification of genetic variants of viruses; c) epidemiological

models to predict severity, duration, and likelihood of transmission of disease; d) models to evaluate the degree of success of control and prevention interventions; e) 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 f) models for surveillance sampling that identify optimal combination of sampling size, frequency, and targeting to maximize the probability of detecting virus circulation rather than disease.

ASSUMPTIONS

The following captures assumptions made by the FMDCWG in assessing potential countermeasures to enhance our ability to contain and eradicate an outbreak of FMD in the United States.

Situation

Countermeasures assessed for worst case scenario: an act of bioterrorism with two different FMDV serotypes released simultaneously on multiple sites, including two sales barns in the Southeast, a dairy cow replacement operation in California, a feedlot in the Southwest, and a cow-calf operation in the Midwest.

Target Population at risk

Countermeasures assessed for the following agricultural segments in priority order:

1. Cow-calf operations
2. Stocker calves
3. Dairies
4. Feedlots
5. Pigs
6. Sows
7. Sheep
8. Goats
9. Wildlife

Scope of Outbreak

- Two FMDV serotypes
- Multiple locations throughout the United States
- Multiple cattle segments: beef and dairy
- Estimated number of cattle affected: 500,000
- Estimated number of contacts: 10,000,000

Vaccine Administration

Federal and State vaccination crews can vaccinate 10 million head of cattle in 4 weeks.

Diagnosis

Pen-side test is available but all test samples must be sent to FADDL, NVSL, and NAHLN FMD-certified laboratories for confirmatory testing.

DECISION MODEL

The FMDCWG used the quantitative Kemper-Trego (KT) decision model to assess available countermeasures. Instructions for using the model were provided to the working group prior to the meeting of April 11, 2007 (see Appendix I). The model was modified by the working group for the purpose of assessing FMD vaccines, diagnostics, and biotherapeutics (See Appendices II, III, IV, V, VI, and VII).

Criteria

The FMDCWG selected core criteria to enable the comparison of countermeasures using a pertinent and valid analysis, as follows:

Vaccines

- Efficacy
- Cross-protection within serotypes
- Cross-serotype protection
- >1 year duration of immunity
- < 1 week onset of immunity
- No maternal antibody interference
- Two year shelf life
- Safe vaccine
- No high containment required
- DIVA compatible
- Rapid scale-up (> 10 million doses)
- Reasonable cost
- Short withdrawal period
- Feasibility of registration
- Add new antigens
- Accelerated delivery

Diagnostics

- Sensitivity
- Specificity
- Validation to purpose
- Speed of scale-up
- Throughput
- Pen-side test/field-based assays
- Rapid result
- Need for a confirmatory test
- Need for serological test to show recovery (absence of circulating virus)
- DIVA compatible
- Easy to perform
- Cost to implement
- Less reliance on complete cold chain

Weight

Each criterion was weighted to allow a quantitative comparison of the impact of the selected interventions (see Appendices).

Product profile

To ensure a consistent and meaningful assessment, the desired product profile (i.e., the benchmark) that would enable the control and eradication of FMD was identified for each countermeasure:

Desired Vaccine Profile

1. Highly efficacious: prevents transmission in all major ruminant species and pigs; efficacy in young animals
2. Cross-protection (cross-protection within serotypes)
3. Cross-serotype protection (cross-protection against all 7 serotypes)
4. One dose with >1 year duration of immunity
5. One week or less onset of immunity
6. No maternal antibody interference
7. Two year shelf life
8. Safe vaccine: non-abortegenic; all species; pure vaccine, lack of NSP contaminants
9. No reversion-to-virulence
10. No high containment required for manufacturing (eliminate need to grow live FMD virus)
11. DIVA compatible
12. Rapid speed of production and scale-up
13. Reasonable cost
14. Short withdrawal period for food consumption (21 days or less)
15. Feasibility of registration (environmental release of a recombinant)
16. Ability to rapidly incorporate emerging viral antigens
17. Less reliance on complete cold chain

Desired Diagnostic Test Profile

1. Direct tests (e.g., antigen, nucleic acid) for control and eradication
2. Indirect tests for post-control monitoring/detection sub-clinical cattle and wildlife
3. Rapid test
4. >95% specificity
5. >95% sensitivity
6. Pen-side test
7. DIVA Compatible
8. Field validated
9. Easy to perform/easily train NAHLN's personnel
10. Scalable
11. Reasonable cost
12. Detect all FMD strains

Values

The values assigned by the FMDCWG for each of the interventions reflect the collective best judgment of FMDCWG members (see Appendices II, III, IV, and V)

ANALYSIS

The protection of herds against FMD has been a concern of livestock producers for centuries. FMD is one of the most contagious infectious diseases known with a complex epidemiological profile that includes several animal species and therefore requires an integrated approach for control and eradication. Paramount is the availability of effective diagnostics for early detection. FMD includes seven serotypes and each serotype has variants or subtypes requiring the availability of homologous vaccine strains for effective immunological prophylaxis. All ungulates are susceptible requiring animal species-specific control strategies. Because trade restrictions impact the uses of vaccines in FMD-free countries, highly effective countermeasures are needed that can prevent virus transmission and not impede diagnostic surveillance. As a result, this analysis focuses on priority countermeasures that will need to be deployed in concert to prevent the spread of FMDV should an outbreak ever occur in North America.

Vaccines

Effective immunological prophylaxis for the control of FMD is probably one of the most complex problems facing animal health authorities worldwide and therefore requires significant background information before an assessment of available vaccines and vaccine technologies can be completed and understood. The following section provides specific information on the history and breakthroughs in FMD vaccine development and a detailed analysis of available commercial and experimental vaccines.

History of FMD Vaccine Development

The early research that went into the development of FMD vaccines contributed significantly to some of the major vaccine discoveries of the 20th century (Lombard M., et al, 2007). The first attempt to develop an FMD vaccine was published in 1926 by French researchers Vallée, Carré, and Rinjard (Vallée H. et al, 1926). Their breakthrough contribution resulted from testing the action of formaldehyde on different agents of infectious diseases and they were the first to report the successful inactivation of FMDV using ground FMD lesions in saline buffer filtered and inactivated at 20°C for 4 to 7 days with 0.5% formaldehyde. Although the resulting vaccine provided inconsistent efficacy it was nevertheless on a par with vaccine standards of the time.

The next breakthrough came from Professor Waldemann and his team at the German Institute of Riems Island in the Baltic Sea in 1937 with the semi-industrial production of FMD vaccine adjuvanted with aluminum hydroxide (Waldemann D., 1937). The major contribution of the German team was the improvement of the inactivation process that highlighted the importance of key criteria, such as ensuring a pH >9 during inactivation, using a lower concentration of formaldehyde (0.05%), and maintaining the inactivated material at a higher temperature (25°C for 48 hours). This was the first modern technology for producing FMD vaccines and it remained the standard for 50 years until the 1970s when attempts were made to use other inactivants.

Once the barrier of successfully inactivating virulent virus was overcome the next challenge was to produce enough vaccine FMD viruses to achieve industrial scale production. The breakthrough came

once again from the Riems Island research team with a method for harvesting large quantities of virus, a process known as the Waldemann's method. The method involved harvesting virulent material from infected cattle held in a restricted stable, inoculated at the same time at several sites on the tongue, and slaughtering the cattle when the lesions are at their worst. Although crude by today's standards, one has to remember that tissue culture did not exist at that time. The virulent tongue materials were ground in saline buffer, centrifuged, and diluted before inactivation. The process resulted in 60 ml of vaccine fluids yielding 40-50 commercial cattle doses per tongue.

The next breakthrough is credited to Professor Frenkel from the Amsterdam Veterinary Institute in Holland who devised the first primary tissue surviving system using epithelial fragments from cattle tongues collected from abattoirs. The process involved maintaining for 48 hours or more in an appropriate medium at 37°C under oxygen bubbling small epithelia tissues infected with a virulent FMD master seed virus. This method yielded 100 times more vaccine virus per animal than the Waldemann method yielding 400 commercial cattle doses.

In 1951, Espinet in Chile discovered that saponins could be used as an effective adjuvant in the aluminum hydroxide gel, which combined with the Frenkel virus production method led to the first FMD vaccine available at commercial scale for vaccination campaigns (Espinet R.G., 1951). To meet the extensive demand for FMD vaccine doses, 500 liter culture tanks were used, which provided larger vaccine batches and reduced the cost of each commercial dose.

The next industrial breakthrough was the use of cells in suspension to meet the demand for millions of doses for FMD vaccination campaigns in South America and Europe. At first cells were primary or secondary kidney cells derived from calves, piglets, or lambs at abattoirs but were eventually replaced by clean cell lines, including the baby hamster kidney cell line (BHK21). Cells were initially grown in tissue culture monolayers using roller bottles but the challenge of harvesting thousands of bottles without contamination led to the culture of cells in suspension, which became the standard for manufacturing massive volumes to meet the demands of FMD vaccination campaigns.

The development of the BHK21 cell line in suspension was accomplished in 1962 in the UK at the Institute for Animal Health Pirbright Laboratory (then the Animal Virus Research Institute). This work was a major achievement in that the production process could now be completed entirely in a closed system, resulting in the first biosecurity measure to prevent the escape of FMDV from manufacturing plants. The major disadvantage was the presence of allergens from cell culture and the significant number of allergenic reactions during vaccination campaigns. It took an additional decade to fine-tune the purification steps so that potent non-allergenic FMD vaccine could be produced without reducing the yield of vaccine virus (Adamowicz P., 1974).

Adjuvants

Although FMD vaccines formulated with aluminum hydroxide provided satisfactory results in European cattle, these vaccines were less effective in pigs. McKercher and his team working in the United States at Plum Island after 1965 successfully determined the attributes of oil adjuvants to increase the potency of FMD vaccines in pigs (Sutmoller P., and Barteling S.J., 2003). These oil-adjuvanted FMD vaccines were subsequently shown to have significant benefits for cattle in South America where cattle breeding was extensive. Benefits of oil-adjuvanted FMD vaccines administered intramuscularly (IM) included protection under a variety of management conditions and appeared to provide longer duration of

protection than the previously used aqueous vaccines (Sutmoller P., and Barteling S.J., 2003). The availability of quality oil-adjuvanted FMD vaccines is in large part credited for the immense success of FMD vaccination campaigns in South America.

Inactivation of FMDV Vaccines

One of the important breakthroughs in FMD vaccine development was the proper inactivation of virulent FMD vaccine viruses. It was known as early as 1948 that inactivation with formaldehyde resulted in vaccines that remained virulent a few days after inactivation (Moosbrugger G.A., 1948). Kinetic studies with formaldehyde clearly showed that this inactivant was less than optimal. Although inactivation was the goal, higher concentrations of formaldehyde and/or longer incubation period could lead to the deterioration of immunogenic structures and impede potency and efficacy. In 1959, the work of Brown and Crick (Brown F. and Crick J., 1959) identified a new family of inactivants, the aziridines, but their use in the vaccine industry did not occur until 1971 (Pay T.W.F. et al., 1971). The real breakthrough is credited to Bahnemann (Bahnemann H.G., 1973), working for PANAFTOSA (Pan American Foot-and-Mouth Disease Center) in Rio de Janeiro, who demonstrated that a simple chemical reaction could convert aziridine to an effective inactivant just before the inactivation process starts. This method was adopted by vaccine manufacturers worldwide, including a double inactivation step for biosecurity, resulting in billions of doses of FMD vaccine produced without one case of failed inactivation reported.

Vaccine Purification

In the 1990s, revolutionary studies on the role of FMD virus non-structural proteins (NSPs) on the immune response led to development of new diagnostics that could differentiate infected from vaccinated animals (DIVA) (FAO, 1998). Vaccines that did not contain NSPs could be used in vaccination programs without affecting the serological diagnosis of infected or carrier animals.

As a result, the purification of FMD antigens has become paramount for manufacturers using BHK21 cells for the following reasons: 1) to remove heterologous proteins with allergenic properties and 2) to remove the NSPs that could interfere with the new serological diagnostic test methods. New laboratory techniques such as chromatography or the use of polyethylene glycol help greatly with downstream purification steps without affecting vaccine potency. Another benefit of adding a high purification process was the ability to concentrate antigens, which could be frozen and stored in vaccine banks as strategic reserve for emergency vaccination. Manufacturers can now create their own antigen and vaccine banks to enable them to respond within a few days to requests for the formulation of multivalent vaccines anywhere in the world.

Differentiating Infected from Vaccinated Animals (DIVA)

Exposure to “structural” FMDV capsid proteins as well as “non-structural” proteins such a RNA polymerase through infection or vaccination will induce the production of antibodies to these proteins. The removal of non-structural proteins (NSPs) from FMD vaccines enables the differential detection of antibodies to the NSPs in FMDV infected animals. Thus, current DIVA strategies for FMD are based on the use of a diagnostic test that can differentiate the detection of antibodies to NSPs in infected versus vaccinated animals.

However, it is important to note that these tests have been developed after the development of current vaccines and not in parallel, and are thus always struggling to provide a definitive result. Development

of the next generation vaccines will allow the concurrent development of companion differential diagnostic assays.

Although the application of current DIVA strategies have been successfully applied on a herd basis, they still have their limitations: There is concern that current vaccines may give rise to some vaccinated animals that could shed FMDV, or lead to some animals becoming asymptomatic virus carriers without seroconverting to NSPs or producing levels of antibodies against NSPs detectable by current assays. Of additional concern is the fact that current vaccines may have residual NSPs (depending on the manufacturing process) that could result in the detection of NSPs antibodies in vaccinated animals, especially if multiple doses of vaccine are applied annually. In addition, the application of current DIVA strategies is dependent on diagnostic tests originally developed to determine FMDV infection and used primarily for surveillance.

Since the ultimate goal of a DIVA strategy is to "vaccinate to live," i.e., be able to enter the food chain and not be destroyed, it is paramount that vaccinated animals exposed to FMDV will not transmit virus or become carriers. There is therefore a critical need for new and improved FMD vaccines and companion diagnostics specifically designed for DIVA and validated for the purpose intended. It is expected that the next generation of FMDV countermeasures will not only include vaccines designed with negative markers consisting of deletions of non-structural protein epitopes, but also include companion antibody detection assays to determine exposure to FMDV, and direct antigen or nucleic acid detecting assays to verify that a vaccinated animal exposed to FMDV is not infected or a carrier animal.

Potency

There are currently several versions of standards for potency testing FMD vaccines (European Pharmacopeia, 2006; OIE Code, 2009). Considering the high variability, low repeatability and reproducibility of the standard test in the European Pharmacopoeia and the OIE Terrestrial Manual, there have been several studies to evaluate the variations associated with the potency of FMD vaccines (Rweyemamu M.M, et al., 1984; Pay, T.W. and Hingley, P.J. 1987; Pay, T.W. and Hingley, P.J. 1992; Mattion, N., et al., 2004), resulting in proposals from the scientific community to improve these tests (Goris N., et al, 2007 and Goris N. et al, 2008: "Foot-and-mouth disease vaccine potency testing in cattle using homologous and heterologous challenge strains: precision of the "Protection against Podal Generalisation" test" *Goris N, Maradei E, D'Aloia R, Fondevila N, Mattion N, Perez A, Smitsaart E, Nauwynck HJ, La Torre J, Palma E, De Clercq K*.)

The potency of FMD vaccines according to the OIE Terrestrial Manual has traditionally been expressed as the number of 50 percent cattle protective doses (PD_{50}) contained in the dose stated on the label. The PD_{50} is determined in a dose response study in 15 cattle at least 6-months of age given primary vaccination of either one full dose, ¼ dose, and 1/16 dose (five cattle per group, including a two cattle non-vaccinated control group) and challenged by the inoculation of 10,000 ID_{50} (50 percent infectious dose) of virulent bovine virus of the same type or subtype as that used to prepare the vaccine. The potency is thus correlated to efficacy against a homologous challenge in cattle obtained from areas free of FMDV. The European Pharmacopoeia requires that each batch of vaccine contains at least 3 PD_{50} per dose of cattle. European Commission directives state that FMD vaccines must exceed the European Pharmacopoeia and should have an observed potency of 6 PD_{50} per cattle dose. The latest revisions of the OIE Code (OIE Code, 2009), also states that 6 PD_{50} per cattle dose is preferred; however, this is not

an absolute requirement due to the acceptance that this would significantly reduce the number of vaccine doses in internationally established FMD vaccine banks. The case for using higher potency vaccines is clear, including greater protection against heterologous strains, a quicker onset of immunity, and increased protection from viral shedding and transmission.

The high variability associated with the PD_{50} test, including overlapping confidence intervals and absence of statistical control, led to the acceptance of an alternative potency test in South America, which is a modification of the PD_{50} test and has now been added to the OIE Terrestrial Manual (OIE Code, 2009). The PPG test (percentage of protection against generalized foot infection or "Protection against Podal Generalisation") includes a group of 16 FMD-seronegative cattle of at least 6 months of age, with the same characteristics described for the PD_{50} test, which are vaccinated with a full vaccine dose by the route recommended on the label. The animals and a control group of two non-vaccinated animals are challenged 4 weeks or more after vaccination with the challenge strain, which is a suspension of bovine virus that is fully virulent and appropriate to the virus types in the vaccine under test by inoculating a total of 10,000 $TCID_{50}$ (50% tissue culture infectious dose), intradermally into at least two sites on the upper surface of the tongue. Unprotected animals show lesions at sites other than the tongue within 7 days after inoculation. Control animals must develop lesions on at least three feet; for routine prophylactic use, the vaccine should protect at least 12 animals out of 16 vaccinated (75 percent).

Potency tests in other target species, such as sheep, goats or buffalo have not yet been standardized. In general, a successful test in cattle is considered to be sufficient evidence of the quality of a vaccine to endorse its use in other species.

The current OIE Terrestrial Manual now includes a prescribed potency test for pigs, which are modifications of the cattle PD_{50} or PPG tests.

Indirect tests such as measurement following vaccination of virus neutralizing antibodies in tissue cell culture, or ELISA antibodies, or serum-protecting antibodies in suckling mice, may also be used to assess the potency of a vaccine provided that a statistical evaluation has established a satisfactory correlation between the results obtained by the test on the relevant vaccine serotype and the potency test in cattle. The EPP (expected percentage of protection) is used to analyze sera from a group of 16 vaccinated cattle to express the probability of an animal being protected by measuring neutralizing, ELISA or protecting antibodies. In a single group of animals given a full dose of vaccine, the mean individual expected percentage protection should be equal to or greater than 75% when 16 animals are used or 70% when 30 animals are used in the experimental group.

Correlation of serum titers and protection developed in Argentina is based on commercially available oil inactivated vaccines produced by the Frenkel or BHK methods; however, these relationships might not be applicable to new generation vaccines (Robiolo et al, 2010a).

For multivalent vaccines, the presence of more than one serotype must not diminish the induction of antibodies against another serotype or the correlation of antibody titer with protection.

Onset of Immunity

Both aluminum hydroxide-adjuvanted and oil emulsion inactivated FMD vaccines have demonstrated protection against disease development in cattle and sheep in various disease models and experimental challenge studies within 4 days after vaccination. Swine vaccinated with oil emulsion vaccines may be protected against low-level challenges within 4 days, but with higher, direct-contact challenges, protective immunity against disease may not develop until 21 to 28 days post-vaccination.

Duration of Immunity

Cattle vaccinated with three doses of aluminum hydroxide-adjuvanted vaccine had reduced clinical disease up to three years after vaccination. Cattle vaccinated with a single dose of oil emulsion vaccine remained seropositive with titers believed to be protective for at least 90 days after vaccination. Swine challenged with low levels of homologous virus up to seven months after a single vaccination displayed no clinical disease.

Neonatal Immunity

Very little is known about neonatal immunity to FMDV. As indicated in the potency testing section above, potency tests are carried out in cattle 6-months of age or older. It is therefore important to study neonatal immune responses to understand the influence of colostral immunity, potential vaccine interference due to maternally-derived immune factors, and the ontogeny of the immune system in susceptible animal species, all of which need to be taken into account to judge onset of immunity, duration of immunity, and the selection of an adequate vaccine.

Vaccine Matching

The new May 2009 version of the OIE Terrestrial Manual (OIE Code, 2009) is available on the OIE web site (http://www.oie.int/eng/normes/mmanual/2008/pdf/2.01.05_FMD.pdf)) and now includes a new section on vaccine matching. A good review of methods for selecting vaccine strains was published by Paton et al., 2005.

Vaccine matching is paramount to a successful FMD vaccination campaign as vaccination against one serotype of FMDV will not cross-protect against another serotype and may also fail to protect fully or at all against other strain subtypes within a serotype. The most direct and reliable method to measure cross-protection is to vaccinate relevant target species and then to challenge them by exposure to the virus isolate against which protection is required. This approach is slow and expensive and the use of *in vitro* alternatives should be considered.

In vitro serological test methods can be used to quantify antigenic differences between FMDV strains and estimate the likely cross-protection between a vaccine strain and a field isolate. However, relatedness' indexes (r) between FMD strains calculated from currently available serology test results may not accurately predict cross-protection, particularly when using lpELISA. Current tests have been developed to assess homologous responses, thus they do not perform equally well for assessment of heterologous protection. There is therefore a critical need to develop new *in vitro* parameters to correlate with *in vivo* cross-protection; e.g., anamnestic T cell responses, IgG subclasses, IgG avidity.

Genetic characterization, phylogenetic analysis, and antigenic profiling using cartography are powerful tools that can also reveal the emergence of new strains and may indicate that an isolate is similar to one for which vaccine matching information is already available.

Some reports have indicated that antigen payload may also play a role in the level of cross-protection that could be provided by a vaccine (Brehm KE et al., 2008: "High potency vaccines induce protection against heterologous challenge with foot-and-mouth disease virus" *Brehm KE, Kumar N, Thulke HH, Haas B)*. However, these observations may not be true for all serotypes, and the biological bases for such results are not clear. Booster doses of vaccine can increase efficacy and the subsequent range of antigenic coverage or cross-protection provided by a given vaccine strain. This has been demonstrated for FMDV (Mattion et al, 2004) and also other diseases (i.e. equine influenza, Daly et al, 2007," Equine influenza vaccine containing older H3N8 strains offers protection against A/eq/South Africa/4/03 (H3N8) strain in a short-term vaccine efficacy study." *Daly JM, Sindle T, Tearle J, Barquero N, Newton JR, Corning S*). In this scenario, however, the onset of full protection, if achieved, might be delayed compared with homologous protection.

FMD Strategic Reserves

Vaccine banks, antigen banks, or strategic reserves, are collections of immunogenic material ready to be used or ready to be rapidly reconstituted into a final vaccine product in an emergency response to a foreign animal disease outbreak in countries previously free of the disease.

The first mention of a strategic reserve was made following the devastating FMD outbreak in the UK in 1967-1968. A high-level commission established by the British government determined that a stock of FMD vaccine should be maintained should a similar outbreak occur again. Because the vaccine was fully formulated, it had to be discarded at the end of its shelf-life.

As a result, several European manufacturers investigated the possibility of storing FMD viruses using proper buffers and preservatives to resist freezing. In 1974, a French manufacturer published the first patented process for the concentration and purification of the FMD virus prior to inactivation (Adamowicz P., 1974). Although a significant improvement, the advantage of establishing strategic reserves using already inactivated bulk antigen rather than virulent viruses to enable the rapid formulation of vaccines became apparent.

In 1979, the United States Department of Agriculture (USDA) established the first large strategic reserve of FMD bulk antigen or vaccine antigen concentrates. This strategic reserve was soon joined by Mexico and Canada and became the North American FMD Vaccine Bank.

In 1985, the International Vaccine Bank was established at the Institute for Animal Health in the UK. This strategic reserve was established as a response to an agreement between the governments of Australia, Finland, Ireland, New Zealand, Norway, Sweden, and the UK.

In the 1990s, the cessation of routine FMD vaccination in the European Community led to a high demand for the establishment of strategic antigen banks for use in the event of the reappearance of FMD. As a result, several governments negotiated contracts with manufacturers to establish their own national reserves. In 1992, the European Union (EU) launched an ambitious program to store several million doses of FMD vaccine representing important strains (Füssel A-E., 2004).

An essential component to the successful establishment of rapidly deployable strategic reserves includes changes in the regulations in the EU. This has led the European Pharmacopoeia to adapt their

procedures regarding the emergency release of vaccines prepared from previously controlled antigens. At the time, standards pertaining to the emergency release of vaccines had not yet been included in the *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals* (*Terrestrial Manual*) published by the World Organisation for Animal Health (OIE) (OIE Code, 2006b).

The main advantage of vaccine banks is the availability of finished vaccine for immediate use in emergency vaccination. The main disadvantage of vaccine banks is the need to renew the stocks at the end of the shelf-life, which is between 12 and 24 months. If new orders are received too late by the manufacturer, there is a gap between the expiration date of the current vaccine bank and the arrival of new stock.

The main advantage of antigen banks is the ability to produce large quantities of FMD vaccines for vaccination campaigns in FMD endemic countries or the control and eradication of outbreaks in previously FMD-free areas. Paramount is the technology for storing deep-frozen inactivated bulk FMD antigens over liquid nitrogen developed over the past thirty years. Freshly manufactured vaccines of sufficient quantity and containing an appropriate homologous vaccine strain (see Figure 1) cannot be produced rapidly enough to meet market demands. When stored frozen over liquid nitrogen (–130°C), concentrated inactivated FMD antigens have a shelf life of more than five years. In the version adopted in May 2006 by the International Committee of the OIE, the FMD Chapter of the *Terrestrial Manual* (OIE Code, 2006a) describes for the first time the storage and monitoring of antigen concentrates.

The technical advantages and disadvantages of antigen banks are summarized in Appendices VIII and IX, respectively.

Summary of Obstacles to Vaccinating Against FMD

- Conventional inactivated FMD vaccines can not be manufactured in the U.S.
- There is concern in both FMD-free and FMD-endemic countries that live virus may escape from manufacturing facilities, as recently occurred in Pirbright, UK.
- Current vaccines provide only serotype-specific protection, so vaccines prepared with at least 7 serotypes/antigens must be available for use.
- Antigen drift within serotypes results in the emergence of field isolates that may not be controlled with older vaccine antigen types.
- Countries have regulatory restrictions as to the strains that are allowed in individual countries. South America and India have significant restrictions and do not have access to all FMDV strains. Lelystad in Holland is the exception and allows all strains. Pirbright in the UK assesses the strains in their bank quarterly but does not change often.
- Antigen drift within serotypes requires ongoing expense to stockpile newly emerging antigens.
- No currently available vaccine provides "sterile immunity," that is, vaccination may not prevent subsequent infection and/or the development of persistent FMDV carriers.
- There is a potential window of vulnerability during the neonatal period: protection induced by commercially-available vaccines, serotypes and combination of different serotypes has not been significantly studied during the neonatal period. The influence of colostral immunity is not known.
- Significant variations in manufacturers' production methods and quality control tests may impact the performance of FMD vaccines in the field. For example, a vaccine with a potency of 3 PD_{50}

per cattle dose may perform quite differently under field conditions than a vaccine with a potency of $\geq$6 $PD_{50.}$

- There are concerns that current vaccines may have residual NSPs that could result in the detection of NSPs antibodies in vaccinated animals, therefore prohibiting the implementation of effective DIVA strategies. Quality control testing for purity can now be standardized by the new filtration-assisted chemiluminometric immunoassay (FAL-ELISA) (Capozzo et al., 2010). This test just became commercially available in a kit format in October 2010.
- Since production methods and quality control tests are considered confidential by the large majority of manufacturers, it is imperative that owners of antigen banks to be vigilant in acquiring FDMV vaccine strains that match the viruses circulating in the field. Quality control testing for purity can now be standardized by FAL ELSIA (Capozzo et al, 2010).
- Although formulation is important, the selection of the correct vaccine strain is paramount.
- FMDV Serotype O is less immunogenic and requires a higher antigen payload than other serotypes.
- FMDV Serotypes SAT 1, SAT 2, and SAT 3 are less stable than the other four serotypes and require additional quality assurance measures to ensure potency throughout the manufacturing process and storage.
- The presence of vaccinated livestock in the U.S herd after disease eradication will extend international trade prohibitions.

Assessment of Commercial Vaccines (See Appendix II)

Congress passed legislation in the 1950's that made it illegal to possess live FMDV on the U.S. mainland; thus, conventional inactivated FMD vaccines cannot be manufactured in the U.S and licensed by the USDA-APHIS, Center for Veterinary Biologics (CVB).

CVB Notice 02-21, "Domestic Manufacture of Biologicals Used in the Prevention or Treatment of Foreign Animal Diseases," does provide for the production and licensing in the U.S of biotechnology-derived FMD vaccines that do not require the production of live FMD virus (see next section, assessment of experimental vaccines).

Based on the evaluation of satisfactory vaccine data (efficacy, potency, purity, and safety), manufacturing methods, production facilities, and final product testing, a biological manufacturer could be issued a U.S. Veterinary Biological Product Permit to import an FMD vaccine for "Distribution and Sale." As of November 5, 2007, the APHIS-Veterinary Services Deputy Administrator has approved CVB to now consider "Distribution and Sale" permit applications for foreign-manufactured conventional inactivated FMD vaccines. As a result, a permit for distribution and sale for the quadravalent (A24, A2001, C3, and O1 strains) vaccine manufactured by Biogenesis-Bago and used in Argentina and elsewhere in South America is expected to be issued to Transboundary Animal Biologics, Inc. (TABI) by January 2011.

Current commercial FMD vaccines consist of inactivated (killed virus) formulated with various proprietary adjuvants formulations. FMD vaccines represent the largest share of the veterinary vaccine market worldwide in terms of sales, representing 26.4 percent of the entire livestock biological business (Gay C.G., et al., 2003). A list of FMD vaccine manufacturers and the vaccines produced worldwide is provided in Appendix X. As discussed in the previous section, significant steps have been made to improve the quality of FMD vaccines, but there are significant differences between different

manufacturers, and vaccines distributed for use in either FMD-endemic regions versus FMD-free countries. Accordingly, acquisition of any commercial vaccine will require an in-depth investigation and due diligence evaluation of the manufacturer and the product for sale to determine the actual profile of the vaccine for the purpose of suitability for control and eradication.

The FMDCWG identified three adjuvant formulations that represent the large majority of all commercial inactivated FMD vaccines worldwide and assessed their value against the desired vaccine profile for FMD control and eradication (See Product Profile on Page 35).

1. **Emergency Use (high potency) FMD Vaccines (NAFMDVB)**

   - In cooperation with Canada and Mexico, the U.S. has assisted in the development of a tripartite "North American Foot and Mouth Disease Vaccine Bank (NAFMDVB)."
   - Vaccines prepared from frozen antigen banks with a potency of at least 6 protective dose 50 (PD_{50}) provide an onset of immunity within 4 days in cattle, swine, and sheep, and provide wider antigenic coverage and protection for heterologous FMDV subtypes within a serotype. Vaccines decrease clinical disease, virus amplification (shed and spread), and the frequency of FMDV carriers.
   - Bulk antigens stored in the U.S must be shipped to South America or Europe for formulation and finishing.
   - Initiating deployment will require at least 3 days for finishing vaccine from frozen antigen concentrates.
   - Several of these vaccines have been extensively tested for early protection and shown to induce protection against challenge by 7 days post vaccination (Golde, Pacheco et al., 2005).
   - Because normal batch or serial tests to demonstrate purity, safety, and potency require several weeks to complete, there has been some concern in the U.S that any use of vaccine prior to the satisfactory completion of these batch tests represents a risk that such product is contaminated, dangerous, or worthless.

2. **Conventional (ready-to-use) Oil Emulsion FMD Vaccines**

   - Oil adjuvanted vaccines prepared with a potency of at least 3 PD_{50} have been shown to provide an onset of immunity within 7 days in cattle, swine, and sheep. Vaccines decrease clinical disease, virus amplification (shed and spread), do not prevent the occurrence of FMDV carriers after FMDV challenge.
   - Differences in efficacy and potency have been reported between double oil emulsion versus water in oil single emulsion formulations (Hunter, P., 1996; Iyer A.V., et al., 2000; Smitsaart E., et al., 2004), with higher and longer antibody response reported for double oil emulsion.
   - Enhancement of the immune response induced by the inclusion of saponin in oil adjuvanted vaccines has been reported (Smitsaart, E., et al., 2000).

3. **Aluminum Hydroxide-Adjuvanted Vaccines**

   - The aluminum hydroxide-saponin FMD originally developed for FMD vaccines has several disadvantages compared with oil emulsion vaccines. Aluminum hydroxide vaccines:
     $\Rightarrow$ are not very effective in swine

⇒ have a shorter shelf life than oil emulsion vaccines
⇒ are less potent per *ug* of antigen
⇒ produce a shorter duration of immunity

Assessment of Experimental Vaccines (see Appendix III)

Since structural proteins are the main antigens responsible for inducing protective responses, several attempts have been made to improve current inactivated vaccines utilizing cloned capsid proteins expressed by rDNA technology. However, these subunit vaccines produced in E. coli or peptide vaccines induce narrow immune responses that the virus evades through the production of quasi-species. Recently, significant improvements in rDNA-based vaccines have been made offering improvements in efficacy, safety, and disease control and eradication.

1. **Human Adenovirus 5 (Ad5)-vectored Adjuvanted FMD Vaccine Platform**

   Since the first gap analysis conducted November 2007, the Ad5-FMD vaccine platform was changed by adding an adjuvant at point of use to increase the potency and efficacy of this vaccine platform.

   ARS-USDA scientists initially discovered that FMD virus particles lacking nucleic acid (empty capsids) are as immunogenic as inactivated vaccines and induce protective immunity (Mayr, Chinsangaram et al., 1999); (Mayr, O'Donnell et al., 2001); (Moraes, Mayr et al., 2002); (Wu, Moraes et al., 2003). This vaccine technology uses an adenovirus vector (Ad5) that contains all relevant portions of the FMDV genome required for empty capsid synthesis and assembly, but lacks the non-structural proteins that allow for easy differentiation of infected from vaccinated animals. The Ad5 platform allows for safe production as the process does not involve the use of live FMDV. In addition, this vaccine platform does not require cell-culture adaptation of field strains thus precluding potential antigenic change during the vaccine manufacturing process. In a series of USDA-ARS pilot studies using a prototype Ad5-FMD vaccine, immunization with one dose of Ad5-FMD vaccine demonstrated full protection against FMD challenge in cattle (Pacheco, Brum et al., 2005). Based on this discovery, a new generation of FMD vaccines are under early and full development in collaboration with DHS and an industry partner, GenVec, Inc. (Brough, et al., 2007; Grubman, et al., 2010). These new vaccine candidates are currently being manufactured in experimental batches on the U.S. mainland (for the first time in U.S history) and tested in cattle at PIADC as part of the veterinary licensing process. Plans are in place to have the first new-generation vaccine licensed and available for use in 2011.

   The Ad5-FMD molecular vaccines under development are based on a replication-deficient human serotype 5 viral-vectored backbone. The human adenovirus C, serotype 5 (Ad5) genome containing deletions in the E1, E3, and E4 regions is replication deficient in target host cells. The E1 region deletion, including the E1A and E1B promoters and open reading frames, renders the adenovirus vector replication deficient. The E3 region is not essential for growth of Ad5 in tissue culture. The E4 deletion eliminates essential elements for Ad5 replication through removal of the E4 open reading frames. Although the Ad5 viral backbone lacks the essential genes required for in vivo replication, it is capable of growing to high titers in specialized tissue culture cell lines (e.g. 293-ORF6, M2A) that contain stable chromosomal copies of these essential adenovirus genes. . The generation of a replication competent adenovirus (RCA) would require two independent recombination events in a single adenovirus genome. Although

the rate of RCA generation has not been determined for the Ad5-FMD molecular vaccines, it is predicted to be extremely rare.

The Ad5 backbone has been used extensively in humans and is being used in FDA Phase I, II, and III clinical trials for various indications. The Ad5-FMD vaccine is classified as a BL-2 agent, thereby allowing production and in vitro characterization in the absence of BSL-3 containment that is required for live FMDV.

Western blot analysis of Ad5-FMD infected cell lysates is used to evaluate in vitro expression and the correct processing of the FMDV capsid precursor polyprotein into the mature VP0, VP3, and VP1, capsid proteins. Although preliminary process development and biochemical and electron microscopy studies have identified the presence of putative empty viral capsids (EVCs) in the adenovirus expression system, additional work is necessary to determine their role in vaccine protection. Process development activities at the 1 liter and 10 liter bioreactor level are currently in progress or planned to identify upstream and downstream production parameters that can be readily transferred to large scale manufacturing.

For cattle safety and efficacy Ad5-FMD vaccine studies, the dose is based on Ad5-FMD expression titer in which the titer is determined using a FMDV-specific monoclonal antibody. At present, experimental vaccines are stored as frozen liquid at -70°C in final formulation buffer (FFB) and administered intramuscularly in a 2 ml dose containing adjuvant. Preliminary stability data demonstrates the Ad5-FMD vaccine virus is very stable, with no significant loss in infectivity after 18 months storage at -20°C.

No evidence of adverse reactions has been observed in any of the clinical trials conducted to date. Based on the lack of seroconversion to either FMD or Ad5 in co-mingled sham-immunized cohorts, vaccinated cattle do not appear to shed Ad5-FMD vaccine virus. Reversion to virulence and backpassage studies on Ad5-FMD vaccine virus have been successfully performed. A study was completed in lactating cattle and no evidence of Ad5-FMD vaccine virus was found.

An Ad5-FMD subtype A24 Cruzerio vaccine is the most advanced product candidate in development. To date, this vaccine has been tested in over 300 cattle. At the appropriate dose, this vaccine has been shown to provide significant protection against generalized FMD disease (> 95% protection) and viremia following a single immunization and IDL (tongue) challenge at either 7 or 14 days post-vaccination. Protection as early as 4 days post-vaccination had been demonstrated in some, but not all animals. In addition, protection against FMDV infection has been observed in a small number of vaccinates based on the absence of tongue lesions following IDL challenge. Sequencing of the capsid region in FMDV isolates obtained from tongue lesions has shown no evidence of the generation and selection of FMDV antigenic variants in vivo following Ad5-FMD subtype A vaccination. Notably, the Ad5-FMD subtype A vaccine has also shown protection in a contact cattle challenge model that is more representative than the IDL challenge to a field outbreak scenario. Pivotal minimum immunizing dose studies have been satisfactorily completed and a vaccine release dose has been approved by USDA CVB.

A preliminary Summary Information Format (SIF) for the Ad5-FMD candidate was submitted to USDA-CVB June 2007 and a Federal Register Notice proposing environmental release for field

safety testing, including an environmental risk assessment, has been published. No significant public comments were submitted. Field safety studies are planned for Spring 2011 and if these studies are satisfactory, all requirements for CVB licensure of the A24 vaccine will be completed, and a conditional license should be issued in 2011.

The Ad5-FMD vaccines induce serotype- or subtype-specific serum neutralization (SN) titers following a single immunization. A portion of immunized cattle have detectable SN titers as early as Day 4 and >50% of vaccinated cattle have detectable anti-FMDV SN titers at 1 week post-immunization. In studies conducted to date, pre-challenge anti-FMDV SN titers tend to peak 14 to 21 days post-vaccination and titers increase following FMDV challenge. Duration of immunity (DOI) studies have not been performed to date, however a minimum DOI of 3 months is anticipated. DOI studies are included in the post-licensing development plan.

The Ad5-FMD vaccines induce anti-adenovirus serotype 5-specific (e.g., anti-vaccine vector) SN titers following a single immunization. In general, some cattle have detectable SN titers as early as Day 4 and all vaccinated cattle have detectable anti-Ad5 SN titers at 1 week post-immunization. In studies conducted to date, anti-Ad5 titers tend to peak 2 weeks post-vaccination and then begin to decline. A pilot study conducted by USDA-ARS showed that a 2nd vaccine dose administered following the decline of the initial anti-Ad5 response can successfully boost anti-FMDV SN titers.

A FMDV 3D ELISA companion test is under development in collaboration with USDA-ARS and USDA-APHIS FADDL. In Ad5-FMD vaccine studies conducted to date, post-vaccination/pre-challenge serum samples test negative in the 3D ELISA. Confirmatory testing (including false positives) in the fully validated Prionics FMDV – NSP ELISA has also been completed with similar results. Additional development of the 3D DIVA test is required to address false positives, and serum samples from all Ad5-FMD vaccine trials will continue to be tested in both assays.

Current Ad5-FMD vaccine candidates in the R&D pipeline include two (2) Ad5-FMD serotype O vaccines, one (1) Ad5-FMD serotype pan Asia vaccine , four (4) additional Ad5-FMD subtype A vaccines and one (1) Ad5-FMD serotype SAT2 vaccine.. Information generated from the development and licensure of the first Ad5-FMD serotype A24 vaccine will be applied to the Ad5-FMD vaccine candidates in the pipeline. Construction, proof-of-concept testing, and development of vaccine candidates for the remaining FMDV serotypes (SAT1, SAT3 and C) and other emerging genotypes are also planned by DHS S&T over the next few years.

Summary Assessment of Ad5-FMD Vaccine

The Ad5-FMD vaccine platform is in the development phase represented by the lead vaccine candidate, Ad5-FMD subtype A24. The Ad5-FMD vaccine platform has several advantages over conventional FMD vaccine platforms, including 1) eliminating the need for BSL-3 containment facilities for vaccine production, 2) lacks the non-structural protein coding regions that allow for easy differentiation of infected from vaccinated animals, 3) safe production process that does not involve the use of live FMDV, 4) does not require cell-culture adaptation of field strains, and 5) precludes potential antigenic changes during the vaccine manufacturing process. Similar to conventional FMD vaccines, the Ad5-FMD vaccine platform provides

serotype-specific and subtype-specific protection against FMDV disease as early as 7 days post-vaccination. USDA-CVB licensing studies using Ad5-FMD subtype A24 vaccine derived from master cell bank and master virus bank were initiated in 2008, and satisfactory pivotal efficacy studies were reported by the manufacturer in September 2010 leading to CVB acceptance of demonstration of a reasonable expectation of efficacy and a vaccine release dose. An adjuvant safety study is planned for early 2011 and if successful, will allow for approval of the Field Safety Study protocol, FONSI issuance and subsequent completion of the final clinical study (Field Safety Study) by summer 2011. Each construct will need to be developed and evaluated fully for purity, potency, safety, and efficacy prior to U.S regulatory approval by the CVB and environmental release under NEPA. This process typically requires 3-5 years to complete for each replication-defective recombinant vaccine.

2. **Double marker cDNA-derived Killed FMDV Vaccine Platform (FMD-LL3B3D)**
USDA-ARS scientists have generated a cDNA-derived foot-and-mouth disease virus (FMD-LL3B3D) as a safe platform for production of a marker inactivated vaccine. This vaccine platform comprises a genetically engineered attenuated FMDV backbone, molecularly and antigenically marked by deletion of the Leader protein and conserved B cell immunodominant epitopes to allow serological differentiation of vaccinated from infected animals. Further modifications are the inclusion of unique restriction endonuclease sites for rapid replacement of capsid coding sequences. Attenuation of the double negative marker virus was achieved by manipulating the genome to eliminate the L^{pro} coding sequence (Leaderless – LL), which is known to be involved in FMDV pathogenesis *in vivo*. Here (A_{24}FMD-LL3B3D) and in previous studies (A_{12}LLV2), it has been shown that deletion of the FMDV leader proteinase coding sequence created viruses that maintained the ability to infect BHK-21 cells but display low virulence for cattle or pigs (Almeida *et al.* 1998. *Virus Res.* 55:49-60; Brown *et al*., *supra*; Chinsangaram *et al*., *supra*; Mason *et al.* 1997, *supra*). Animals infected with FMD-LL3B3D by the aerosol route (cattle) or by direct inoculation in the feet (swine) demonstrated that the prototype virus candidate is highly attenuated for clinical disease and failed to spread to contact animals in both susceptible livestock models. This platform also allows for rapid exchange of capsids of different serotypes and utilizes cell culture and manufacturing procedures currently used by FMD vaccine production companies but allows for a simplified downstream processing without the need to eliminate the viral non-structural proteins. This feature could result in higher antigen yields, higher vaccine potency, and reduce the cost of production. Inactivated, oil adjuvanted, vaccines, consisting of chemically inactivated FMD-LL3B3D virus, showed an efficacy comparable to a polyvalent commercial FMDV vaccine and protected 100% the animals from challenge with parental virus (Rieder *et al.* in preparation). The double marker vaccine candidate induces immune responses similar to those obtained from inactivated wild type virus, except that the vaccine virus lacks critical antigenic epitopes in the non-structural viral proteins (3B and 3D) recognized by specific monoclonal antibodies directed to 3B and 3D proteins from FMDV but not to other closely related picornaviruses. Serum from animals infected with the vaccine virus can be readily distinguished from parental-FMDV infected animals utilizing serological tests such as competitive enzyme linked immunoabsorbent test (cELISA).

Summary Assessment of double marker cDNA-derived Killed FMDV Platform (FMD-LL3B3D)
This platform is currently under development by USDA-ARS scientists at the Plum Island Animal Disease Center. The FMD-LL3B3D platform has several advantages over conventional

FMD vaccine platforms and has the following attributes: 1) the engineered negative markers provide the means for specific and sensitive detection of FMDV in the recovery phase of an FMD outbreak, allows the differentiation of infected from vaccinated animals when vaccination is used as a control measure, eliminates the need to remove non-structural proteins which should lower costs of good, and the presence of non-structural proteins may improve the quality of the vaccine immune response against FMD viruses; 2) the removal of the leader protein sequence resulting in the attenuation of the virus, thus mitigating concerns associated with intentional or accidental vaccine escapes from a manufacturing plant; 3) the strategically-located restriction-enzyme sites that allow easy swapping of the relevant antigenic region for different serotypes and subtypes, allowing rapid incorporation of emerging field strain antigens into the vaccine production platform; 4) the FMDV strain used as the backbone for this vaccine platform was selected for its ability to grow efficiently in the BHK cell lines used for production by FMD vaccine manufacturers, thus minimizing the lost of vaccine virus titers during production resulting from the engineered deletion and mutations; and 5) this vaccine platform can fit in any of the currently used FMD manufacturing production systems (including inactivation and adjuvantation) and thus eliminates the need and costs associated with designing and validating new manufacturing methods. Although this new vaccine platform is still at the discovery phase, these attributes and safety and efficacy data generated to date resulted in this vaccine platform being rated above all other experimental vaccines evaluated by the FMD Countermeasures Working Group (See Appendix III).

3. FMD Peptides

In lab animal models (mice, guinea pig), several FMD capsid based peptide vaccine candidates have been shown to induce peptide-specific and anti-FMDV SN titers, and in some instances have been shown to confer protection against FMDV challenge. Unfortunately, these positive results in lab animal models have not been consistently reproduced in cattle and pigs.

In a large-scale synthetic peptide vaccination study in 138 cattle using 4 different FMDV serotype C VP1 G-H loop based peptides, none of the peptides, tested at several doses and vaccination schedules, conferred protection above 40% (Taboga, Tami et al., 1997). Notably, several mutant FMDV strains were isolated from vaccinated cattle, suggesting that peptide vaccination induced the rapid generation and selection of FMDV antigenic variants in vivo.

Efforts to improve and broaden VP1 G-H loop peptide immunogenicity through the incorporation of T helper (Th) sites and incorporation of consensus residues into the hypervariable positions ("UBI peptide") resulted in high level of protection in swine following FMDV 01 Taiwan challenge (Wang, *et al.*, 2002). A subsequent pilot study in cattle showed that the UBI peptide induced peptide-specific antibodies but relatively low SN titers, and failed to protect cattle following FMDV type O challenge at 3 weeks post-vaccination (Rodriguez, Barrera et al., 2003)

FMDV peptide vaccine adjuvanted with cholera toxin and administered transcutaneously elicited anti-peptide antibodies with enhanced virus neutralizing activity in mice ((Beignon, Brown et al., 2005), however further experiments in target species are still required. Recent studies in swine utilizing non-toxic *Pseudomonas aeruginosa* exotoxin A expressing the FMDV VP1 G-H loop failed to induce protective immune responses (Challa, Barrette et al., 2007)

The recent development of dendrimeric peptides containing one copy of an FMDV T-cell epitope branching out into four copies of a B-cell epitope provides potential improvements over conventional linear peptide (Cubillos et al., 2008). Pigs vaccinated with adendrimerric peptide and subsequently challenged with FMDV did not develop significant clinical signs, appear to have abrogated systemic and mucosal FMDV replication, and prevented transmission to contact controls. The dendrimeric peptide used in this experiment elicited an immune response comparable to that found for control FMDV-infected pigs. Dendrimeric designs for other FMDV serotypes and subtypes need to be developed and tested but this new technology provides substantial promise for peptide subunit vaccine development.

Summary Assessment of FMD Peptides

To date, there have been no reports of successful FMD peptide efficacy in cattle and there are very limited reports of protective effects in swine. The likely requirement for multiple peptide vaccine doses and the relatively slow onset of protective immunity does not fit with the target product profile for stockpiling and emergency use FMDV vaccines. Moreover, none of the leading FMDV research centers are actively working on FMD peptide vaccines and significant basic research is still required.

4. Alternative VLP Platform

Virus-like particles (VLPs) are non-replicating, non-pathogenic particles that have structural characteristics and antigenicity similar to the parental virus. They are similar in size and conformation to intact virions and are formed by the self-assembly of envelope and/or capsid proteins. The structural components of some VLPs have also proven amenable to the insertion or fusion of foreign antigenic sequences, allowing the production of chimeric VLPs exposing the foreign antigen on their surface.

There are several expression systems for the production of VLPs, including (1) various mammalian cell lines, either transiently or stably transfected or transduced with viral expression vectors, (2) the baculovirus/insect cell system, (3) various species of yeast including *Saccharomyces cerevisiae* and *Pichia pastori*, and (4) *Escherichia coli* and other bacteria.

A yeast-derived VLP experimental FMD vaccine was initially described almost 15 years ago (Balamurugan, *et al.*, 2003). The capsid P1 gene from a serotype O strain induced SN and ELISA titers in guinea pigs and these animals were protected against homologous challenge. More recently, co-expression of either recombinant bovine interferon-gamma (Shi, *et al.*, 2006), IL-18 (Shi, *et al.*, 2007) or HSP-70 (Su, *et al.*, 2007) and VLP P1 constructs has been shown to enhance SN and CMI responses in mice, however no livestock vaccine efficacy studies have been reported.

Baculovirus-derived VLP experimental FMD vaccines can afford some protection against clinical disease in swine, but fail to elicit strong protect against viral replication (Grubman, *et al.*, 1993). Similar results using an *E. coli*-derived VLP experimental vaccine were also reported (Grubman, *et al*., 1993). More recently studies using recombinant silkworms baculovirus for the

expression of P12A-3C coding sequences of an Asian FMDV strain has shown reasonable levels of protection in cattle (li et al 2008). In this study and after challenge with the homologous Asian virus, four of five vaccinated animals were completely protected. Current efforts using the baculovirus system are also directed toward the production of recombinant FMDV antigens for diagnostic tests.

Hepatitis B virus core (HBc) particles, self-assemble into capsid particles and are extremely immunogenic. However, formation of VLPs can be restricted by size and structure of heterologous antigens. The first report of using the HBc system for expression of amino acids 141-176 of the VP1 protein of FMDV was made 20 years ago, and the immunogenicity of the VLP structures was reportedly similar to that of intact FMD particles (Clarke, *et al.,* 1987). Very recently, the formation of VLP in mammalian cells by modified HBc fused with specified FMDV multi-epitopes was studied. Complete VLP structures with one construct were confirmed by electron microscopy and induced both humoral (peptide- and FMDV-specific antibody) and CMI (IFN-γ, IL-4) responses in mice (Zhang, *et al.,* 2007).

The generation of VLP experimental FMD vaccines using transgenic plants has also shown some laboratory success. *Arabidopsis thaliana* transformed plant extracts expressing the FMDV VP1 gene were shown to provide protection against FMDV challenge in mice (Carrillo, *et al.,* 1998). Similar studies have also been reported using transgenic potato plants (Carrillo, *et al.,* 2001) or alfalfa plants (Wigdorovitz, *et al.,* 2005) as immunogens in serology and challenge studies in mice. Related studies using HBc to express a VP1 capsid epitope in transgenic tobacco has also been reported (Huang, *et al.,* 2005). To date, none of these transgenic plant derived VLP experimental vaccine candidates has been efficacy and safety tested in cattle or swine and the regulatory and manufacturing path for transgenic plant-derived vaccines is not well defined.

Summary Assessment of Alternative VLP Platform

This platform is still in the Discovery phase and requires more time and effort to advance a candidate into DHS targeted advanced development. The majority of the VLP experimental FMD vaccines constructed to date have not been tested for efficacy in cattle or swine, and those that have been tested have shown only partial protection. Among the various VLP expression systems, mammalian cell culture systems are favored for appropriate modifications and authentic assembly of VLPs, but are a less controllable system and more costly for production. Thus, manufacturing considerations are likely to limit the practical utility of many VLP approaches for FMD vaccines, while the small size of vaccine antigens that can be incorporated into some VLPs may also prove to be a significant barrier to vaccine efficacy.

5. Species-specific Adenovirus-Vectored FMD Vaccines

Several replication-competent or replication-defective nonhuman adenoviral vectors have been developed and investigated for their potential as vaccine vectors (Bangari and Mittal, 2006). Bovine adenovirus serotype 3 has been used to produce experimental vaccines for bovine herpesvirus type 1(BHV-1) (Reddy, *et al*., 2000) and bovine viral diarrhea virus (BVDV)(Baxi, *et al.,* 2000). Porcine adenovirus serotype 3 has been used to produce experimental vaccines for transmissible gastroenteritis virus (TGEV)(Reddy, *et al.,* 1999), classical swine fever virus (Hammond, *et al*., 2000) and pseudorabies virus (Hammond, *et al*., 2001). Similarly, porcine

adenovirus serotype 5 has been used to produce an experimental vaccine for TGEV (Tuboly, *et al.*, 2001). Unfortunately, none of these experimental vaccines have been developed and licensed for veterinary use, so additional work on these vectors is required, with particular emphasis on the focus on the safety aspects associated with the replication-competent vectors. Although there has been no basic research performed on bovine or porcine adenovirus vectors co-expressing FMDV structural and 3C protease genes, there is no scientific reason to believe this approach will not work. However, specialized cell lines required for vaccine vector production will need to be identified. These vectors will need to be constructed and characterized, and then compared against the human Ad5-FMD vaccine platform in safety and efficacy studies in cattle and pigs.

Summary Assessment of Species-specific Adenovirus-vectored FMD Vaccines

Limited safety and efficacy studies have been completed to date using species-specific Ad-vectored FMD vaccines. None of the leading FMD research centers are actively working on this approach and significant basic research is still required.

Diagnostics

The FMDCWG determined that the effectiveness of available diagnostics is high but several obstacles need to be addressed to ensure diagnostics are available, strategically deployed, and used effectively. Table 1 summarizes the most relevant diagnostic tests that are available now or under development. The U.S diagnostic system for detecting FMDV consists of strategic links between the National Animal Health Laboratory Network (NAHLN) and the Federal Foreign Animal Disease Diagnostic Laboratory (FADDL), USDA-APHIS, Plum Island, Orient Point, New York. The mission of the NAHLN includes surveillance, response (surge), and recovery from high consequence agricultural diseases. High-throughput semi-automated robotic systems have been deployed and are utilized in the NAHLN laboratories. These systems are compatible with sample processing and rapid nucleic acid detection technologies. The following section provides current obstacles for detecting FMDV, specific information on tests for vaccine matching, diagnostic strategies (surveillance, response, and recovery), assessing herd immunity, and a detailed analysis of available commercial and laboratory tests as well as assays under development (for comparative assessment see Appendices IV, V, VI, VII, and VIII). Manufacturers of commercial diagnostic test kits are provided in Appendix X.

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Obstacles for Detecting FMDV

- Very few Veterinarians have observed FMD lesions
- Farmers and cattle producers are not trained to recognize FMD
- FMD in small ruminants is often subclinical or may look like foot rot
- Non-FMD oral lesions have frequently been observed in sheep
- FMD can look like other vesicular diseases and laboratory testing is required for confirmation
- Effective laboratory and field systems are required and should be in place prior to an outbreak of FMD. These systems play a critical role in the overall management and control of the outbreak and include:
  - Trained field staff (disease recognition and immediate response)
  - Collection and storage of field data from cases
  - Cross training, diagnostic contingency planning, and table top exercise
  - Transport of material
  - Sample type for testing (lesion, blood, milk, probang, swabs)

- Unpacking and processing of samples
- Laboratory information management system (LIMS)
- Reporting of results to decision makers

- Rapid scaling to meet high influx of samples in face of an outbreak.
- Penside tests have not been field validated and policies on their use are not in place

1. Surveillance

The first line of defense against an FMD outbreak relies on reporting of suspicious cases by personnel handling or observing susceptible animals such as farmers, technicians, farm hands, and veterinarians. A certified Foreign Animal Disease Diagnostician (FADD) investigates suspicious cases and ships appropriate samples to the Plum Island Foreign Animal Disease Diagnostic Laboratory (FADDL), USDA-APHIS, and in occasion to the local certified NAHLN for rapid screening.

1.1 Clinical diagnosis

Infection of susceptible animals with FMDV typically results in vesicles on the feet, in and around the oral cavity, and on the mammary gland of females. Vesicles may also occur inside the nostrils or – especially in pigs – at pressure points on limbs. Severity of clinical signs may vary from sub-clinical to severe, based upon the strain of FMDV, the exposure dose, the host species, host age and breed, and host immune status. Multifocal myocarditis may be observed, especially in young animals. However, there is great variability in clinical signs depending on the viral strain, the age of the infected animals, and the animal species.

1.2 Tests to detect infected animals

Tests are needed to rapidly detect cases in the field and confirm positives in the laboratory. Pen-side tests can be a powerful tool if appropriately distributed to trained veterinarians in the field. In initial suspect cases, there may be an additional requirement to perform differential diagnosis from other diseases causing vesicular lesions in livestock, such as Vesicular Stomatitis (VS), Vesicular Exanthema of Swine (VES), and Swine Vesicular Disease (SVD). Although many of the rapid assays initially used to detect FMDV do not identify the specific serotype causing the outbreak, other assays are used for this purpose during later stages.

1.2.1 Molecular assays

Available now

- Lab-based semi-automated RT-PCR - This realtime RT-PCR assay for FMDV is extremely rapid with a total turnaround time of less than 2 hours after the samples have been processed (Callahan et al., 2002). The assay was used as a frontline diagnostic test during the 2007 FMD outbreak in England (Reid et al., 2009). The assay has been shown to detect all known serotypes of FMDV with sensitivities equal to or greater than virus isolation. It has been shown to have a limit of detection of between 10 and 100 viral genome copies per sample. Diagnostic validation of the assay has indicated that the overall sensitivity and specificity is:

Total Samples Tested = 3,243

Parameter	Cutoff Ct Value ≤40	Cutoff Ct Value ≤45
Diagnostic Sensitivity	93.75%	96.34%
Diagnostic Specificity	99.93%	99.57%
Accuracy	99.04%	99.11%

The assay is easy to perform although it requires RNA extraction and strict protocols to prevent contamination. The assay amplifies only a very small portion of the FMDV genome and is not applicable to virus strain characterization or serotyping.

In development

- Multiplex RT-PCR assays for vesicular disease "rule-out" - Multiplex bead-based assays for using the Luminex format are currently in the early stages of feasibility testing. These assays allow the simultaneous detection of FMDV and other vesicular look-alike viruses (SVDV, VESV, VSV, BVDV, etc.) that cause vesicular disease in livestock. These assays are being developed as species specific panels for bovine and porcine. Multiplexed diagnostic assays such as this are clearly needed by diagnostic laboratories to streamline testing and improve efficiency; however, the added cost associated with testing numerous other disease targets during an outbreak or in recovery from an outbreak is not feasible. The assay in its currently published configuration does not demonstrate the needed sensitivity for FMDV detection. A modified set of primers and capture probes have recently been developed by Lawrence Livermore National Laboratories to improve the sensitivity for FMDV detection however this new configuration needs validation.

- Microarrays have the capacity to identify FMDV by genotype, without any prior characterization of the suspected agent. This technique may be useful where directed diagnostic methods such as PCR are unable to produce a definitive result due to sequence heterogeneity, or genome mutation. Non-biased random amplification of nucleic acid from samples is performed on test sample, and then bound to the microarray. Bioinformatics analysis of positive features allows for identification of viral genotype, as well as characterization of specific regions of viral genome sequence to aid in identification. Advantages to this system include the ability to test samples of unknown genome sequence, and the capacity for multiplexing,

1.2.2 Serological assays

Available now

- ELISA tests to detect antibodies against NSP - most of them are indirect species-specific tests (UBI, Svanova, Panaftosa) and only the Priocheck (blocking ELISA) allows detection in multiple species. Comparative performance of these tests has been published (Brocchi et al., 2006). In naïve animals exposed to infection the sensitivity of all ELISAs for 3 susceptible species (cattle, swine and sheep) was almost 100%.

In development

- FMD NSP 3ABC ELISA- Next generation NSP 3ABC ELISA that uses a 3ABC recombinant protein and a monoclonal specific for an immunodominant B-cell epitope that can be used when conventional or Ad5 marker vaccine (absence of 3B gene) is being developed and will be evaluated and validated for detection of antibodies to nonstructural proteins of the FMDV in infected animals regardless of their vaccination status.

- FMDV NSP 3D antibody ELISA – A liquid phase blocking 3D ELISA has shown promising preliminary results in early detection of antibody against FMDV in both bovine and swine (FADDL– unpublished data). Additional next generation competitive 3D ELISA under development uses a 3D protein expressed in E. coli and virus-specific monoclonal antibodies for detection of antibodies against the FMDV NSP (Rieder personal communication). These assays are not only capable of detecting antibody against all FMD serotypes but can also be utilized as a companion test for the Ad5 empty capsid viral vaccine which is missing the 3D protein. These tests could be used in support of a vaccinate-to-live policy as they can discriminate between infected and non-infected animals regardless of their vaccination status. It is also important to note that seroconvertion against 3D protein occurs 2-3 days earlier than that to 3A, 3B and 3ABC proteins which makes the 3D ELISA superior to other nonstructural protein assays in regards to early detection. The 3D protein has been being used in AGID test as the complementary test in sero-epidemiological studies. Recombinant 3D protein has been expressed in E. coli and baculovirus culture. Development and full validation of a 3D ELISAs are still in the development phase.

2. Response

2.1 Tests in the early stages of an outbreak

These assays are low-throughput and are performed in the initial phases of an outbreak for characterization of the field strain responsible for the epizootic to determine the serotype and strain characteristics.

2.1.1 Molecular assays

Available now

- Strain characterization by nucleotide sequencing - RT-PCR amplification of FMD virus RNA, followed by nucleotide sequencing, is the current preferred option for generating the sequence data to perform strain characterization. Many laboratories have developed techniques for performing sequence analysis of the P1 or VP1 region of the FMD genome.

In development

- USDA-APHIS-FADDL has developed a simple universal P1 RT-PCR in collaboration with the Institute for Animal Health (IAH), Pirbright, UK, that is in preparation for publication (Lizhe Xu, et al., unpublished).

- USDA-APHIS-FADDL is also developing genotyping microarrays and interpretive software and these may also serve as a rapid screens for genotyping FMDV (Barrette, et al., unpublished).

2.1.2 Antibody-based assays

Available now

- Antigen ELISA for serotyping (using polyclonal or monoclonal reagents) - The vesicular antigen ELISA for the detection and identification of vesicular disease causing viruses was developed by Crowther and Abu Elzein in 1979. The assay was evaluated and/or modified by a series of investigators (Ouldridge, *et. al.* in 1984, Hamblin, *et. al.* in 1984, and Have, *et. al.* in 1984). Final improvements were made by Roeder and Le Blanc Smith in 1987, and the assay was validated by Ferris and Dawson in 1988 for use at the European and World Reference Laboratory (EWRL) for FMD (Pirbright Laboratory, England). Ferris and Dawson proved the vesicular antigen ELISA to be a superior test, due to increased sensitivity and reproducibility, economical use of reagents, and ease of performance when compared to the complement fixation test (CFT). The assay was later slightly modified and validated by Dulac *et. al.* in 1993 for use at the Animal Diseases Research Institute (ADRI) in Canada. The procedure involves an initial capture of rabbit antiserotype antibodies on a 96 well ELISA plate, followed by an incubation with the sample (tissue homogenate, vesicular fluid, and cell culture isolates). The antibody-antigen reaction, if it occurred, is detected by the addition of guinea pig antiserotype detector antibodies, followed by the addition of conjugate, substrate, and stop solution.

2.2 Tests for vaccine matching

Effective and efficient tests for "vaccine matching" are critical to determine and predict the expected efficacy of available FMD vaccines. Appropriate vaccine strain selection is a critical element in the control of FMD and is necessary for the application of vaccination programs in FMD affected regions as well as for the establishment and maintenance of vaccine antigen concentrates to be used in the event of new FMD incursions (OIE Manual, 2004). There are seven serotypes of FMDV and approximately 65-70 subtypes. Vaccination against one serotype of FMDV does not cross-protect against another serotype and depending on the serotype, one vaccine may not protect against all of the subtypes within a particular serotype. Given the variety of serotypes and subtypes of FMDV circulating in countries where FMD is endemic, an effective response will require rapid serotyping/subtyping of the outbreak strain and subsequent matching to vaccines contained in the NAFMDVB. The most direct method for determining vaccine cross-protection is an *in vivo* experiment in which the target species is vaccinated and subsequently challenged with the field isolate. However, this is time consuming and expensive. *In vitro* alternatives for vaccine matching include the two dimensional neutralization test, ELISA and sequence analysis of the P1 region of the FMD genome. Sequence analysis by itself cannot predict differences in antigenicity and therefore needs to be backed up by structural information combined with serological/protection data. This is an area of research that is still under development, as stated below.

Serological matching of field isolates to vaccine strains requires that isolates have been serotyped and adapted to growth in cell cultures. The serotype is usually determined by ELISA or CFT using type-specific serological reagents, although methods based on monoclonal antibodies or genetic typing may also be used. BHK or IB-RS-2 cell cultures are usually used for *in vitro* virus replication. For vaccine matching, preferably, at least two isolates should be evaluated from any outbreak and inconsistent results should be followed up to determine whether this is due to genuine antigenic differences or is an artifact of testing.

The serological relationship between a field isolate and a vaccine virus ('r' value) can be determined by CFT, ELISA or VNT. One way testing is recommended (r_1) with a vaccine antiserum, rather than two way testing (r_2) which also requires an antiserum against the field isolate to be matched. Due to the inherently low repeatability of the assays used, tests need to be repeated to be confident of the results (55). *In vitro* neutralization may be more relevant to *in vivo* protection than other measures of virus-antibody interaction, although non-neutralizing antibodies may also be protective. Advantages of ELISA are that the test is rapid and utilizes smaller volumes of post-vaccination sera which are often available in only limited quantities. ELISA and CFT are recommended to be used as screening methods whereas the VNT method provides more definitive results. For either VNT or ELISA, post-vaccination sera should be derived from at least five cattle 21–30 days after immunization. The titer of antibody to the vaccine strain is established for each serum and samples may be used individually or pooled, after excluding low responders (Mattion et al. 2009).

2.2.1 Antibody-based assays

Available now

- Vaccine matching by Liquid Phase Blocking ELISA (LPBE) - This test uses an antiserum raised against a vaccine strain. The blocking ELISA titres of this reference serum against antigens prepared from the homologous vaccine strain and are compared with the corresponding titres of the serum against a field isolate to determine how antigenically 'similar' the field virus is to the vaccine virus.

- Vaccine matching by two-dimensional neutralisation test - This test uses an antiserum raised against a vaccine strain. The titres of this serum against 100 $TCID_{50}$ of the homologous vaccine strain and the same dose of a field isolate are compared to determine how antigenically 'similar' the field virus is to the vaccine strain. Required biological reagents are: 21–30 day post-vaccination bovine vaccine sera (inactivated at 56°C for 45–60 minutes); the homologous vaccine strain; and the test virus, a field isolate of the same serotype as the vaccine strain.

In development

- Antigenic cartography - One of the difficulties in controlling FMD comes from the wide diversity that exists among the seven different serotypes of FMDV and the additional subtypes that exist within these. Vaccination against one of these serotypes does not cross-protect against the other serotypes and often not even subtypes within the same serotype, making vaccine matching by serological means necessary. However, the antigenic relationships between field viruses and how they may have

evolved is not easily determined by this method since the serological relationships are inconsistent and dependent on individual sera. In an effort to improve vaccine selection, antigenic cartography is being used to interpret serological data in order to visualize and quantify the relationships between strains.

- Antigenic profiling - The antigenic profile resulting from the reactivity of field strains with panels of monoclonal antibodies raised against vaccine strains of the same serotype is thought to be a promising method for vaccine matching (Mahapatra et al., 2008). Monoclonal antibodies have the advantage of being able to be well characterized, standardized and replenishable reagents, characteristics not shared with the polyclonal serum used for the vaccine matching by neutralization.

  The testing method used for antigenic profiling is a capture ELISA in which pre-titrated viruses are captured with type specific rabbit polyclonal antibodies, followed by incubation with MAb or guinea pig polyclonal antibodies. The reactivity is detected with rabbit anti mouse or anti guinea pig HRP conjugate and substrate. The percentage reactivity of each monoclonal antibody compared to that of the guinea pig polyclonal is calculated. Values of 20% or greater are considered positive and a formula could be used to calculate the percentage antigenic homology of a particular field isolate to the parent vaccine virus.

  The implementation of antigenic profiling has not been very successful so far in its correlation with the gold standard vaccine matching method of virus neutralization. In order to improve its performance a wide number of well defined monoclonal antibodies needs to be included representing each antigenic site on the surface of the viruses. A more thorough understanding on the contribution of neutralizing and non-neutralizing epitopes in protection is required to determine which MAb to include in the panel, and equalization of the amount of the virus captured in the ELISA plates needs to be optimized. Antigenic profiling has the potential of becoming a fast and reliable method for vaccine matching.

2.2.2 Molecular assays

Available now

- Vaccine matching by nucleotide sequencing - RT-PCR amplification of FMD virus RNA, followed by nucleotide sequencing, is the current preferred option for generating the sequence data to perform strain characterization. Many laboratories have developed techniques for performing sequence analysis of the P1 region of the FMD genome; however, sequence analysis by itself cannot predict differences in antigenicity and therefore needs to be backed up by structural information combined with serological/protection data.

- Methodologies are now available for complete genome sequence analysis that may become beneficial to comparisons of field strains and determining relationships to existing vaccines.

2.3 Tests for early and sustained response

Continue with the lab-based semi-automated (r)RT-PCR assay used during surveillance (1.1 molecular assays described above).

2.4 Assays for detection of FMDV-infected animals:
To be used in initial suspect cases. There may be an additional requirement to perform differential diagnosis from other diseases causing vesicular lesions in livestock. Testing capability should encompass the possibility of use for targeted surveillance for "dangerous contacts" and epidemiological links to clinical cases (e.g., spread of infection in buffer zones). The diagnostic tests used to detect FMD infected animals are based on cellular bioassays (virus isolation), or detection of viral proteins (Antigen ELISA) or nucleic acids (RT-PCR).

2.4.1 Bio-assays

Available now

- Virus isolation in cell culture – Virus isolation is considered to be the "gold standard" method for the detection of FMD. This approach can be highly sensitive and highly specific (when used in combination with antigen-ELISA to confirm the presence of FMDV after CPE is observed). However, there are considerable differences between the cell lines routinely used by the different National FMD Laboratories. Primary bovine thyroid cell cultures have been shown to be the most sensitive for field strains of FMD although sourcing these cells can be problematic particularly in the face of an outbreak. The capability to perform virus isolation is essential for antigenic characterisation of field isolates and is a critical step in the preparation of conventional vaccine seed stocks.

2.4.2 Antigen detection assays

Available now

- Antigen ELISA for serotyping (using polyclonal or monoclonal reagents) – Antigen capture ELISAs using FMDV-specific immunological reagents (polyclonal antisera or characterised monoclonal antibodies: Ferris and Dawson, 1988) are rapid diagnostic assays suitable for confirming FMD in suspect cases. However, these assays are less sensitive than virus isolation (and rRT-PCR) and are therefore not suitable to confirm the absence of disease on a farm. Specific reagents to perform these tests should be generated, characterized and stockpiled prior to an outbreak.

- Lateral-flow devices for rapid detection of virus in the field - LFDs (also known as "dip-sticks") are simple-to-use "point-of-care" tools that can be used for first line diagnosis by Veterinarians in slaughter houses, farms or in simply equipped regional laboratories (Ferris et al., 2009 and 2010) and http://www.svanova.com. These devices (e.g., SVANODIP® FMDV-Ag) offer the potential to rapidly feedback "real-time" information to decision-makers, ensuring that control policies are most closely matched to the risks of disease spread in the field. In addition it is possible to extract RNA from these devices to be used in PCR for confirmation and further

characterization of the virus (King and Bankowski, personal communication). Devices for FMDV antigen detection have been extensively tested in the laboratory and some in the field and are available from commercial sources. The device is being evaluated at PIADC-FADDL on fresh clinical samples representing the major FMDV serotypes and subtypes to determine the window of detection during the course of infection in bovine and swine, to measure diagnostic sensitivity in endemic countries and the diagnostic specificity in the US animal population.

2.4.3 Serological assays

Available now

- Lateral-flow devices for FMDV-specific antibody (Structural Protein and/or NSP - LFDs to detect NSP antibody are available from commercial sources in China and South Korea, however, these devices have not yet been sufficiently validated for use. These devices should allow for detection of antibodies to all seven serotypes of FMD virus.

In development

- Additional lateral-flow devices for FMDV-specific antibody (Structural Protein and/or NSP) - LFDs to detect NSP antibody are also under development and evaluation. In a similar manner to the LFDs for antigen detection (described above), these devices are designed for the simple field detection of NSP. Devices under development are utilizing in vitro expression of NSPs as foundation for LFD. The NSP or NSPs will be bound to the chromatographic strip and subsequently bind FMD antibodies from the samples in question. These devices should allow for detection of antibodies to all seven serotypes of FMD virus.

- *Multiplex NSP Luminex assay*- Liquid array technology allows simultaneous measurement of the relative responses of multiple signatures to a challenge sample. The use of such multiplexing technology has time, cost and manpower benefits over multiple, singleplex analyses, in addition to an increased confidence in results. The benefits of these assays have yet to be realized. Multiple signature evaluation provides more confidence when obtaining a conclusive result, it eliminates variations that may occur when using a series of singleplex assays to obtain a comparative result, and it allows controls in every sample. The liquid array consists of beads that are embedded with precise ratios of red and infrared fluorescent dyes yielding 100-bead sets, each with a unique spectral address. Analyte that is captured on a modified bead is detected using a detector reagent, indirectly labeled with a fluorescent reporter. Each optically encoded and fluorescently labeled bead is then interrogated by a flow cytometer. A classification laser (635 nm) excites the dye molecules inside the bead and classifies the bead to its unique bead set. A reporter laser (532 nm) excites the bound fluorescent reporter and quantifies the assay at the bead surface. The flow cytometer is capable of reading around one hundred beads per second; analysis can be completed in as little as 15 s and potentially up to 100 different analytes can be assayed simultaneously, thereby providing a high-throughput and economic platform. Serological assays such as this will be of considerable value to

diagnostics particularly if they target the same sample matrix such as serum or meat juice. Such assays may target Abs to different pathogens or serotypes as well as different isotype specific responses to the same pathogens. Optimization of specificity and background correction in results has been traditional challenges associated with this technology. Such assays should be developed to be DIVA compatible.

The current FMD Mx DIVA Assay contains 4 non structural protein signatures: 3A peptide, 3B peptide, 3D peptide and 3ABC recombinant protein plus 4 controls – instrument control, fluorescent control, antibody control and negative control. The 3ABC signature in the multiplex shows comparable performance to a widely used commercially available assay, and in addition, the multiplexed assay provides a large amount of extra information about the relative diagnostic sensitivity of each signature in one experiment. This feature of the multiplexed assay is particularly attractive when considering the potential use of the assay in vaccine development and assessing vaccine purity.

2.4.4 Molecular assays

Available now

- Lab-based semi-automated RT-PCR - It is now recognized that RT-PCR assays can play an important role for the rapid and sensitive detection of FMDV in a wide range of clinical sample types. Recent development of real-time RT-PCR methodology employing a fluorescently labeled probe to detect PCR amplicons has allowed the diagnostic potential of molecular assays to be realised. These assays are highly sensitive and obviate tube opening after amplification thereby reducing the potential for cross-contamination of test samples by post-PCR products. In order to increase assay throughput and minimize operator errors, rRT-PCR assays for FMDV can be automated using robots for nucleic acid extraction and liquid handling equipment to set-up the reaction mixes. Together with the implementation of quality control systems, these improvements have increased the acceptance of the rRT-PCR assays for statutory diagnostic purposes. Although on-going studies continue this work, there is already a wealth of data that focuses on different aspects of validation to support the use of rRT-PCR for routine FMD diagnosis (King et al., 2006; Shaw et al., 2007; Hoffmann et al., 2009).

  rRT-PCR assays for FMDV are extremely rapid with a total turnaround time of less than 2 hours following sample preparation. The assay can be utilized for surveillance and confirmation, and was used as a stand-alone diagnostic assay during the 2007 FMD outbreak in England (Reid et al., 2009). The assay has been shown to detect all known serotypes of FMDV with sensitivities equal to or greater than virus isolation. The assay is easy to perform although it requires RNA extraction and strict protocols to prevent contamination. The assay amplifies only a very small portion of the FMDV genome and is not applicable to virus strain characterization (Callahan et al., 2002).

In development

- Mobile/portable equipment (rapid diagnosis in non-centralized laboratory) - The time taken to transport suspect material to a centralized laboratory can be unacceptably long, often precluding laboratory confirmation in the event of an outbreak. Using existing available equipment, there are opportunities to deploy mobile rRT-PCR assays inside a vehicle, or in local laboratories, for rapid diagnosis of suspect cases. Previous studies (Hearps and others 2002; Callahan and others 2002) have developed rapid reverse-transcription polymerase chain reaction (RT-PCR) assays for FMDV detection; however, limitations in the hardware and some aspects of the protocols used have restricted the adoption of these assays for the field detection of FMDV. A number of equipment platforms that offer simple-to-use RNA extraction protocols for use by non-specialists (such as veterinarians or field technicians) are currently under evaluation for this purpose (King et al., 2008).

- Isothermal methods - Loop mediated amplification (LAMP) amplifies specific nucleotide sequences. However, unlike PCR, a denatured template is not required and DNA is generated in abundance so that positive LAMP reactions can be visualized with the naked eye. LAMP has been reported to have equivalent (or improved) analytical sensitivity when compared to PCR methods for virus detection. A prototype one step, single-tube, accelerated, RT-LAMP assay has been developed for FMDV (Dukes et al., 2006). This assay is able to detect all seven FMDV serotypes and is capable of detecting 10 copies of FMDV within 25 minutes. Amplification products can be detected by visual inspection, agarose gel electrophoresis, or in real-time by the addition of a fluorescent dye. This method may have potential use as the basis of a highly sensitive, extremely rapid, specific, and cost-effective device for field use.

- Multiplex RT-PCR assays for vesicular diseases - Multiplex bead-based assays for using the Luminex format are currently in the early stages of feasibility testing. These assays allow the simultaneous detection of FMDV and other vesicular look-alike viruses (SVDV, VESV, VSV, BVDV, etc.) that cause vesicular disease in livestock. APHIS-FADDL is currently developing species specific panels bovine and porcine but is still in the early feasibility stages.

- DNA chips (Micro arrays) - Microarrays ("DNA chips") have the capacity to perform numerous assays on the same sample material. The utility of this format to allow detection and high resolution characterization of FMD present in samples is under evaluation. Currently, these assay formats are too slow and expensive for routine use during or in recovery from an outbreak, though costs continue to drop. Multi-pathogen, pan-viral, or pan-pathogen microarrays are best suited to detection of unanticipated or highly divergent foreign animal, zoonotic or emerging diseases. They are typically based on non-biased random RT-PCR or in-vitro transcribed amplification of sample RNA and as such is ideal for screening complex disease syndromes. A handful of comprehensive pan-viral or pan-pathogen arrays have been developed and published including the ViroChip (Wang, et al., 2003), the Greene

Chip (Palacios et al., 2007), the FADDL panviral array (Barrette, et al., 2009), and the microbial detection array (Gardner, et al. 2010).

- TIGER: The TIGER (Triangulation Identification for the Genetic Evaluation of Risk) biosensor is a highly sophisticated technology with unparalleled capabilities for detecting, identifying, and "fingerprinting" high consequence emerging, zoonotic and agricultural pathogens. This newly developed biosensor combines the powerful tools of PCR, mass spectrometry and bioinformatics in order to detect and perform high resolution fingerprinting of infectious disease pathogens. The unique attributes of this technology when compared to those currently in use include: the ability for high resolution "fingerprinting" of known, unknown, zoonotic and emerging pathogens (NOTE: does not depend on the availability of sequence data for organism in question); the ability to monitor through "high resolution" fingerprinting, genetic drift, virulence and mutations within the pathogen of interest; and the ability to detect multiple pathogens within one sample.

2.4.5 Screening technologies

In development

- Infrared Thermography (IRT) – IRT has been extensively used for mass screenings of airline passengers during the SARS and H1N1 Influenza outbreaks (Ng EYK, 2004. Microvascular Research 68: 104– 109) and in the early detection of breast cancer, herpes labialis and SARS in humans. In veterinary medicine IRT has been previously used for detection of orthopaedic problems, contaminated ear implants in calves (Spire MF, et al 1999) and bovine respiratory disease (Schaefer et al. 2007).

  One of the main problems hampering the diagnosis, control and eradication efforts during an FMD epidemic is the need for veterinarians to inspect hundreds and in some cases thousands of individual animals in suspected case premises. This is particularly difficult since many animals present mild clinical signs that required close examination of the mouth and each foot. Pen-side rapid diagnostic tests would be instrumental in the early detection of FMD but the selection of animals to test requires time-consuming close examination. In the absence of overt clinical signs, rapid screening is necessary to select likely infected animals for further testing.

  An often observed sign of FMD is the presence of fever although certain species and certain viral strains cause only mild or no fever and when present can be of short duration. Increased temperature has also been observed on the hooves of cattle with FMD vesicles. . This increased heat is most likely due to an inflammatory response surrounding FMDV-affected tissues and the measurement of this thermal energy could assist veterinarians in identifying the appropriate animals in a herd setting for diagnostic testing (see Figure 3). Previous work at the Plum Island Animal Disease Center (PIADC) showed that the use of IRT allowed detection of FMD infected cattle 24-48h prior to the onset of clinical signs (Rainwater et al, 2009).The sensitivity of this approach is between 61 and 72% and specificity is near 90% (using 2 FMDV serotypes: A and O) in correctly identifying FMDV infected animals prior to the

development of clinical signs (Rainwater-Lovett et al., 2009). Another recent study also done at PIADC reports the use of IRT to detect FMDV-infected mule deer (Dunbar et al 2009. J Zoo Wildl Med. 40(2):296-301). Despite these promising observations, IRT detection of FMDV-infected animals requires further research particularly under field conditions. Also the devices tested at PIADC were expensive research apparatus. However, less expensive commercial versions are available: http://www.flirthermography.com/cameras. Recent work at IAH indicated that it will be difficult to determine baseline cut-off points for individual animals. Foot temperatures are greatly affected by ambient temperature and activity (e.g. lying down on straw). In temperate climates, thermal image derived hoof temperatures can only be used to indicate an inflammatory condition such as FMD by reference to the other feet of an animal and its herd-mates and not on the basis of a simple comparison to a threshold for normality (Gloster et al., personal communication). Once field validation is completed, these devices could be used by State veterinarians to select potentially infected animals among large herds during surveillance.

- Air samplers – Simple-to-use air sampling devices have been developed for military and civilian surveillance and disaster-response scenarios. These might be useful as the basis of a non-invasive sampling device for detection of suspect cases of FMD particularly in enclosed places where large number of animal congregate such as auctions, feedlots or large dairies. Detection of FMDV in aerosols has been achieved using a variety of instruments and methodologies. A recent study compared the use of various instruments for FMDV aerosol detection and showed that detecton could be achieved using liquid based air samples followed by RNA extraction and real time rt-PCR can detect FMDV aerosols (Doel et al. 2007; Ryan et al., 2009). ARS researchers at Plum Island made serial collections with two aerosol sampling units: a dry filter PSU from the BASIS/Biowatch programs (http://www.jpeocbd.osd.mil/) and SASS liquid sampler (http://www.resrchintl.com/sass3000-air-sampler.html) during the course of FMDV vaccine trials or pathogenesis studies using FMDV serotypes A, O or Sat 2. Baseline air samplings were carried out for 48 hours before inoculation and at 24 h intervals after infection. Filter and liquid samples were extracted with commercial RNA isolation kits, and real-time RT-PCR was performed using the test developed by ARS. FMDV RNA was detected both in liquid and filter samples starting at 24 h post inoculation and for at least 2 and 3 days post challenge. This time coincides with the onset of nonspecific clinical signs (fever, malaise, mild nasal discharge) but precedes visible vesicular lesions. Both liquid and dry filter aerosol sampling are capable of detecting FMDV in aerosols generated by infected animals and are viable options for real-time surveillance efforts in the event of an outbreak. The application of these techniques in open stables with uncontrolled airflow still needs to be validated, but could be used as a screening method prior to using other diagnostic methods.

2.5 Assays for detection of FMDV exposed animals:

Assays that measure the immune response of an exposed animal. Depending upon policy, the ability to discriminate vaccinated from infected animals may be important. Infection with

FMDV will induce antibodies against structural proteins (SP) and non-structural proteins (NSP), whereas, vaccination with purified, good quality FMD vaccine will only induce antibodies to SP.

2.5.1 Serological assays

Serological tests for FMD are of two types; those that detect antibodies to viral structural proteins (SP) and those that detect antibodies to viral nonstructural proteins (NSPs).

2.5.1.1 Antibodies to structural proteins

Available now

- Laboratory-based VNT, SPCE, and LPBE for SP antibody - The SP tests are serotype-specific and detect and quantify antibodies elicited by vaccination and infection. Examples are 1) the virus neutralization test (VNT) (Golding et al., 1976), 2) the solid-phase competition ELISA (SPCE) (Mackay et al., 2001; Paiba et al., 2004), and 3) the liquid-phase blocking ELISA (LPBE) (Hamblin et al., 1986; Hamblin et al., 1987) and the Single Dilution Liquid Phase Blocking ELISA (SDLPBE), validated to assess herd immunity (Robiolo et al. 2010b). These tests are serotype-specific and are highly sensitive, providing that the virus or antigen used in the test is closely matched to the strain circulating in the field. They are the prescribed tests for trade and are appropriate for confirming previous or ongoing infection in non-vaccinated animals as well as for monitoring the immunity conferred by vaccination in the field. The VNT requires cell culture facilities, the use of live virus and takes 2–3 days to provide results. The ELISA tests are blocking- or competition-based assays that use serotype-specific polyclonal or monoclonal antibodies, are quicker to perform and are not dependent on tissue culture systems and the use of live viruses. Low titer false-positive reactions can be expected in a small proportion of the sera in either the SPCE or LPBE tests. An approach combining screening by ELISA and confirming the positives by the VNT minimizes the occurrence of false-positive results. Reference sera to standardize FMD SP serological tests for some serotypes and subtypes are available from the Reference Laboratory at Pirbright.

- Prionics O ELISA Priocheck - A commercial Solid Phase Blocking ELISA (SPBE) is available but only for serotype O antibodies. PrioCHECK® FMDV Type O is currently the only commercially available serotype specific ELISA on the market.
  The PrioCHECK® FMDV Type O is SPBE is for the detection of antibodies against the FMD virus serotype O in the serum of cattle, sheep, goats and pigs. In this test the plates are coated with the FMDV type O purified-inactivated antigen. The key reagent is an anti-FMDV specific monoclonal antibody (mAb) conjugated to an enzyme that generates a color signal. Binding of the conjugate to the immobilized antigen is blocked by anti-FMDV antibodies present in the test sample. The signal is then measured and when no color is formed, the antibodies in the sample have competed for the viral protein coated on the plate. With this result, the sample is positive for FMDV type O.

2.5.1.2 Antibodies to non-structural proteins

- Laboratory-based NSP testing - Antibody to expressed recombinant FMD virus non-structural proteins (NSPs) (e.g. 3A, 3B, 2B, 2C, 3ABC) can be measured by different ELISA formats or immunoblotting. These assays are used as screening tests and need a confirmatory system, consisting of either a confirmatory assay, or a follow-up of epidemiological units showing results positive at the screening test, or a testing system with known performance. A recent comparison of the currently available NSP ELISAs has indicated that the 3ABC commercial kit from Cedi-diagnostics has the best performance (Brocchi et al., 2006).

- The PrioCHECK® FMDV NS was developed by Prionics affiliate Prionics Lelystad in collaboration with the Danish Veterinary Institute for Virus Research and is the only FMDV NS ELISA on the market that can be used for all species.

- Enzyme-linked immunoelectrotransfer blot assay (EITB) - The EITB assay has been widely applied in South America as a confirmatory test for the NSP serology screening methods (described above). However, the EITB is a difficult test to use and requires experienced staff for interpretation.

- Lateral-flow devices for FMDV-specific antibody available from commercial sources described in 2.4.3 above could also be used.

3. Recovery

3.1 Tests to demonstrate absence of infection

The recovery phase of an outbreak in an FMD-free region requires highly specific tests to demonstrate the absence of FMDV. Different tests are needed depending on whether a non-vaccination or a vaccination strategy was used to eradicate the virus.

If a non-vaccination strategy is utilized the solid-phase competition ELISA (SPCE), liquid-phase blocking ELISA (LPBE) and virus neutralization (VN) tests are appropriate for confirming previous or ongoing infection in non-vaccinated animals. These tests are serotype-specific and are highly sensitive, providing that the virus or antigen used in the test is closely matched to the strain circulating in the field. The VN test requires cell culture facilities, the use of live virus and takes 2–3 days to provide results. The ELISA tests are blocking- or competition-based assays that use serotype-specific polyclonal or monoclonal antibodies, are quicker to perform and are not dependent on tissue culture systems and the use of live viruses. Low titre false-positive reactions can be expected in a small proportion of the sera in either ELISA test. An approach combining screening by ELISA and confirming the positives by the VN test minimises the occurrence of false-positive results. Reference sera to standardize FMD SP serological tests for some serotypes and subtypes are available from the Reference Laboratory at Pirbright.

3.2 Tests to differentiate infected from vaccinated animals (DIVA tests)

If a vaccination strategy was implemented then tests to differentiate infected from vaccinated animals (DIVA tests) would be needed. Infection with FMDV will induce antibodies against

structural proteins (SP) and non-structural proteins (NSP), whereas, vaccination with purified, good quality FMD vaccine will only induce antibodies to SP. Thus, current DIVA strategies for FMD are based on the use of a diagnostic test that can differentiate the detection of antibodies to NSPs in infected versus vaccinated animals. This differential antibody response to FMDV NSPs provides the basis for implementing a DIVA strategy. However, these assays were developed for epidemiological screening of the disease and thus are not fit-for-purpose as DIVA tests.

The amount of NSP produced during infection might not be sufficient to elicit detectable anti-NSP antibodies since some vaccinated animals may suffer subclinical infections (carrier state) with very limited virus replication. Although the carrier state has been documented and studied in vaccinated domestic cattle (Alexandersen, Zhang et al. 2002; Kitching 2002), transmission of FMD has never been convincingly demonstrated under controlled conditions (Sutmoller, Barteling et al. 2003). Furthermore, the antibody response to 3ABC in vaccinated animals that become infected can be very weak or non-existent (Kitching, 1998: van Roermund et al. 2010). Thus, tests to demonstrate the absence of infection need to be highly sensitive; however, the lack of detection of anti-NSP antibodies in individual vaccinated animals does not necessarily mean the absence of infection. This limitation is one of the key elements supporting non-vaccination policies in certain FMD-free countries where test and slaughter policies may be favored to return to market as soon as possible. On the other hand, those supporting "vaccinating to live" policies understand that vaccination may mask viral circulation but question the epidemiological relevance of identifying carrier animals. Accordingly, the vaccinating to live concept and its epidemiological relevance for FMD control is still under discussion in FMD-free countries.

The 3D ELISA is a blocking ELISA for the detection of antibody against the FMDV 3D protein in serum samples of cattle and pigs, potentially other animal species. It is a rapid diagnostic test for FMD with a 4 hr turnaround time for a set of 40-80 samples. Antibody to FMDV 3D protein has been widely used as a diagnostic marker for FMD by a traditional VIAA AGID (agarose gel immuno-diffusion) test. VIAA AGID test, however, is limited by a long turnaround time. To overcome this limitation, a liquid phase blocking ELISA with a 4 hr turnaround time was developed. In this assay, binding of 3D protein to a reporter antibody leads to a color product. Antibody to 3D is determined by the ability of a serum sample, showing a reduced color product once being added to an assay reaction, to block the reporter antibody-3D binding. 3D ELISA, as a diagnostic test, is suitable in area where no vaccine (which can have 3D contamination) or a 3D-minus recombinant vaccine is used. As most of the ELISAs, the 3D ELISA can be adapted to a high throughput assay. One person can perform testing between 400 and 800 samples in an 8 hr working day through automation of the test.

The Prionics **PrioCHECK® FMDV NS** ELISA is a blocking ELISA. It is designed to detect antibodies against the non structural 3ABC protein of FMDV. The wells of the strip plates have been coated with 3B specific monoclonal antibody (Mab) followed by incubation with a baculovirus-expressed 3ABC protein. The test sample is added to the test plate and washed after incubation. FMDV 3ABC antibodies, if present in the sera, will bind to the 3ABC protein coated on the plate and block the binding of the conjugated anti-3B Mab. If there were no 3ABC antibodies present, then the conjugated Mab would be bound. After addition of the chromogen/substrate, color development will indicate the binding of the conjugated Mab indicating the lack of 3ABC antibodies. Consequently, the lack of color development would

indicate the blocking of the conjugated Mab indicating the presence of 3ABC antibodies in the sera (Sorensen et al., 1998).

As mentioned above, these tests have been adopted as DIVA but developed for other purposes, and are thus always struggling to provide a definitive result. Development of the next generation vaccines will allow the concurrent development of companion differential diagnostic assays. A new 3D competitive ELISA currently under development is designed to detect antibodies against the non structural 3D protein of FMDV and to enhance the assay specificity by reducing cross-reactivity with other viruses (Rieder personal communication). This test provides a suitable DIVA test to the Ad-FMDV and for vaccines lacking antigenic determinants in the viral NSPs.

3.3 Tests to monitor herd immunity

Validated assays that have been correlated with vaccine protection are needed.

The SP tests are serotype-specific and detect antibodies elicited by vaccination and infection; examples are the VN test, the SPCE and LPBE. These tests are serotype-specific and are highly sensitive, providing that the virus or antigen used in the test is closely matched to the strain circulating in the field. They are the prescribed tests for trade and are appropriate for confirming previous or ongoing infection in non-vaccinated animals as well as for monitoring the immunity conferred by vaccination in the field. Examples of these tests being used to estimate herd immunity have been published recently (Maradei et al., 2008; Mattion et al., 2009; Robiolo et al., 2010b).

3.4 Tests to detect carrier animals

The serological NSP tests indicate that an animal has been infected (within their sensitivity and specificity). However, these tests cannot distinguish carrier from non-carrier cattle. Currently, the only way to detect carriers is to take regular oro-pharyngeal scrapings and perform VI and/or rt PCR. The results are often inconsistent since it seems that virus is not always present in these samples, time consuming and not suitable for post outbreak surveillance when vaccination was used to control an outbreak.

Parida et al (2006) developed an ELISA to detect IgA in infected cattle since it was postulated that IgA levels will be elevated only in carrier cattle. In their study none of the vaccinated animals had detectable IgA levels 14 days post vaccination. Only carriers, whether vaccinated or not, tested positive for IgA and saliva gave more consistent results than probang and nasal fluids. They also found that IgA in saliva correlated with persistence of virus or viral RNA in OP fluids but provided more consistent results. In contrast the CEDI NSP test provided more consistent results over time, but only carriers developed lasting IgA responses (Parida et al., 2006). Mohan et al. (2008) showed similar findings when cattle were infected with an Asia-1 virus. The current assay is serotype specific, in contrast with the NSP assays that can be used for any serotype. Further developmental and validation is needed before these assays could be used to distinguish carriers with certainty, but the potential is worth supporting. It is also possible to use the IgA tests in conjunction with the NSP assays to detect infected vaccinated animals at higher sensitivity (Parida, presentation at EUFMD).

4. Post-outbreak surveillance

The road to recovery of free status following an FMD outbreak is detailed in the OIE Terrestrial Animal Health Code, Chapter 8.5 FMD (OIE Code, 2009) and requires that the whole territory or part of it is free from FMDV infection/circulation. OIE define virus circulation as transmission of FMDV as demonstrated by clinical signs, serological evidence, RNA presence or virus isolation.

It is essential that high-throughput serological assays are available for use to enable confirmation of freedom from disease after a FMDV outbreak. There is a need for clear sampling strategies and confirmatory test to rule-out false-positives to establish that the whole territory or part of it is free from FMDV infection/circulation. The OIE expect submission of a dossier in support of the application that not only explains the epidemiology of FMD in the region concerned but also demonstrates how all the risk factors are managed. This should include provision of scientifically-based supporting data. There is therefore considerable latitude available to Members to provide a well-reasoned argument to prove that the absence of FMDV infection (in non-vaccinated populations) or circulation (in vaccinated populations) is assured at an acceptable level of confidence.

With this remit the use of either SP or NSP serological tests depending on the vaccination status of the animals to be tested should be suitable tools. Therefore, the tests available are of the same type as described above, 3ABC ELISA or SPCE, but should be used with re-defined sensitivity and specificity fit for purpose. Confirmatory testing of positive samples with VNT or follow-up clinical examination of the suspect animal, probang sampling and rRT-PCR is recommended. High-throughput serological assays are required to confirm freedom from disease after an FMDV outbreak.

5. New diagnostic platforms with potential FMD applications

5.1 Enzyme immune assay membrane tests

This ‘dry ELISA’ format is available for detecting influenza antigen (BD Directigen™ Flu A+B; http://www.bd.com/ds/productCenter/256050.asp). The test is performed on a membrane and results are available within ~15 minutes and is suitable for clinical samples. However, the negative results need to be confirmed in the laboratory. All the reagents necessary for such a device are currently available and just need to be put into the test format.

5.2 Rapid microchip based electrophoretic immunoassays

This device concentrates and detects swine influenza virus on a microfluidic chip with integratred nanoporous membrane. The method relies on separation of labelled ab from ab/ag complex and detection via laser-induced fluorescence. The swine influenza test that has been developed is more sensitive, has reduced assay complexity and time, has a 6 minute reaction time and uses only 50ul sample volume (Reichmuth et al, 2008). Like above, all reagents for FMD detection are available and could be put into this format.

5.3 Allosteric biosensors

This assay is based on a B-galactosidase allosteric biosensor that demonstrates decreased enzymatic activity when foreign peptides are inserted and is reactivated/increased activity in the presence of Mabs or sera from infected pigs. When using optimised reaction conditions, the biosensor could be used to perform DIVA (Sanchez-Aparicio et al. 2009).

5.4 Molecular imprinting techniques on quartz crystal microbalance

This device is based on a functionalized material using molecularly imprinted polymers (MIP) that interacts noncovalently with the analyte - an artificial antibody to the virus that recognises the whole virus particle. Jenik et al (2009) used MIP on human rhino virus (HRV) and found it could distinguish HRV serotypes whether they were denatured or in native form as well as discriminate FMD from HRV.

Biotherapeutics

The FMDV incubation period can be as short as 2 days and animals can shed virus prior to signs of generalized disease. Since FMD vaccines generally require at least 7 days for protective, adaptive immunity to develop, it is critical that FMD control programs include rapid measures to limit and control disease spread. Biotherapeutics or immunomodulators offer the potential to be used as an emergency use tool to stop viral shed and spread within 12 hours after administration and elicit a sustained anti-FMDV effect until the onset of vaccine-induced protective immunity (~168 hours).

Pretreatment of cells with IFN-α/ß can dramatically inhibit FMDV replication ((Ahl & Rump, 1976) (Chinsangaram, Piccone et al., 1999;Chinsangaram, Koster et al., 2001). USDA-ARS scientists showed that at least two IFN- α/ß stimulated gene products (ISGs), double-stranded-RNA-dependent protein kinase (PKR) and 2',5'oligoadenylate synthetase (OAS)/RNase L, are involved in this process (Chinsangaram, Piccone et al., 1999); (de los Santos, de Avila Botton et al., 2006). Based on these observations, USDA-ARS constructed an Ad5 vector containing the porcine IFN-α gene (Ad5-pIFN-α) that produced high levels of biologically active IFN in infected-cell supernatants. Swine inoculated with a single dose of Ad5-pIFN-α were completely protected when challenged with FMDV 1 day later (Chinsangaram, Moraes et al., 2003). The level of protection correlated with Ad5-pIFN-α dose and the level of plasma IFN-α. Additional studies demonstrated that Ad5-pIFN-α treatment alone can protect swine from challenge for 3 to 5 days and can reduce viremia, virus shedding and disease severity when administered 1 day postchallenge (Moraes et al., 2003). Importantly, a combination of Ad5-pIFN-α and Ad5-FMD vaccination can provide both immediate and long-term protection in swine ((Moraes, Chinsangaram et al., 2003); (de Avila Botton, Brum et al., 2006). A similar study in swine was recently reported in which plasmid DNA delivered porcine IFN-α co-administered with a recombinant FMD peptide vaccine provided complete protection following FMDV challenge 5 weeks post-vaccination (Cheng, Zhao et al., 2007).

USDA-ARS scientists have also discovered that type II IFN (pIFN-γ) has antiviral activity against FMDV in cell culture and that, in combination with pIFN-α, it has a synergistic antiviral effect (Moraes, de los Santos et al., 2007). In swine efficacy studies, a combination of Ad5-pIFN-γ and Ad5-pIFN-α, at doses that individually did not protect, induced complete protection in all animals (Moraes *et al.*, 2007). The results indicate that the combination of type I and II IFNs act synergistically to inhibit FMDV replication in vivo. Furthermore, the animals in this group did not have detectable viremia or virus in nasal swab specimens and did not develop antibodies against the viral NS proteins, suggesting that these

animals were sterily protected. More recently this group has started to examine the molecular mechanisms of IFN-induced protection and has found a correlation between protection and both, specific interferon stimulated gene upregulation and tissue specific infiltration of dendritic cells and natural killer cells (Diaz-San Segundo et al., 2010). This information may aid in developing a more robust strategy to induce rapid protection in both swine and cattle.

Recently USDA-ARS scientists demonstrated that Ad5-pIFN-α can sterily protect swine challenged 1 day postadministration with either of 3 different FMDV serotypes, ie., A24 Cruzeiro, O1 Manisa, and Asia-1 (Dias et al., 2010). In addition, swine were protected when challenged 1 day later by either direct inoculation or contact with infected animals. The Ad5-pIFN-α protective dose can be reduced 20-fold when the animals are inoculated subcutaneously at multiple sites in the neck as compared to intramuscular inoculation at 1 site in the rear limb.

In cattle studies, administration of Ad5-pIFN-α failed to completely protect the animals from FMDV infection, although disease was delayed and less severe compared to nontreated controls (Wu, Brum et al., 2003). Recent studies by DHS S&T at PIADC have also shown that Ad5-pIFN-α failed to provide rapid onset of protection, either alone or in combination with an inactivated FMD vaccine (Neilan *et al*, 2006). The reason for the observed lack of efficacy in cattle using Ad5-based IFN constructs in cattle is presently not known and is the subject of ongoing research at USDA ARS. Possible explanations include insufficient Ad5- pIFN-α dose levels required for sustained IFN-α plasma levels in cattle when compared to swine, a role for other type I IFN genes, or the inability of Ad5- pIFN-α to induce downsteam bovine host effector molecules directly involved in the anti-FMDV response.

Summary Assessment of Biotherapeutics

Proof-of-concept efficacy studies using Ad5-pIFN-α in swine have demonstrated its potential as a FMDV biotherapeutic. The recent efficacy studies with Ad5-pIFN-α in a swine contact challenge model, its efficacy against additional FMDV serotypes, and its enhanced potency have increased the development product potential for this platform in swine. Continued basic research on the molecular mechanisms of IFN-induced protection, FMDV pathogenesis and disease resistance in cattle is required to identify lead biotherapeutics or immunomodulators, which can induce very rapid and sustained protection and provide rapid protection in cattle.

This platform is currently in the USDA-ARS Discovery phase and requires additional time and studies to identify a lead candidate for DHS targeted advanced development.

Delivery Devices

As important as having effective vaccines and biotherapeutics is an efficient delivery system for mass vaccination and mass treatment of livestock. Current needle inoculation methods present a challenge to effectively deliver vaccine in the face of an outbreak.

Several needle-free vaccine delivery devices are currently on the market, including the Pulse™ Micro Dose Injection System (Pulse Needle Free Systems), DERMA-VAC™ NF Transdermal Vaccination System (Merial), IDAL® Vaccinator (Intervet), and Agro-Jet® (MIT, Canada). A list of manufacturers and a description of commercially available devices is provided in Appendix XI.

Disinfectants

Sodium hydroxide (2%), sodium carbonate (4%), and citric acid (0.2%) have been reported to be effective disinfectants for FMDV. Less ideal disinfectants include iodophores, quaternary ammonium compounds, hypochlorite, and phenols, because they rapidly lose the ability to disinfect in the presence of organic matter. Surfactants alone have little efficacy against FMDV due to the non-enveloped structure of the virus. There are newer disinfectants that are not as corrosive, including Virkon-S®, a chlorinated compound.

During the outbreaks in the United Kingdom last decade, 0.2% citric acid was successfully used to disinfect environmental surfaces at animal production facilities. In order to further characterize the effectiveness of disinfectants to treat FMDV dried on various surfaces, the Environmental Protection Agency (EPA) contracted research with ARS at the PIADC from 2008-2010. Results from these studies demonstrated are that FMDV dried on either stainless steel or polystyrene surfaces was completely inactivated by 1% citric acid and 1000 ppm sodium hypochlorite after a ten-minute contact time at 22°C. Lower concentrations of citric acid (0.1% and 0.5%) or hypochlorite (500 ppm) failed to completely inactivate FMDV. 4% Sodium Carbonate was able to reduce the titer of FMDV by greater than 4 logs but was unable to completely inactivate the stock of virus, which averaged a titer of greater than 6 logs in recovery controls (recovered in a mixture of disinfectant and neutralizer).

ARS is currently developing a standardized method for testing chemical disinfectants against FMDV using white birch as a porous test surface. Birch was selected because of its similar porosity to pine, yet it does not induce the cytotoxicity associated with pine. Recovery (without disinfection) of dried FMDV from the porous birch veneer coupons has been successful, with a mean virus recovery of greater than 5 logs. Preliminary results suggest that 2% citric acid can completely disinfect dried FMDV on birch surfaces. Sodium hypochlorite has not been effective in these experiments, even after extending the contact time up to 30 minutes and increasing the hypochlorite concentration to 1500 ppm. This is possibly due to inactivation of the disinfectant by the wood surface.

RECOMMENDATIONS

The FMDCWG Chair recommends the implementation of the following research, education, and extension objectives to advance our ability to rapidly detect, control and respond to an FMD outbreak in the United States.

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

- 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.
- 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
- Support research on the immunological mechanisms of cross protection in susceptible species
- Determine the role of cellular innate immune responses in FMDV infection of cattle and swine

- 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 IFNα and β as well as porcine IFNβ
- Contract the development of antibodies to surface markers of critical immune bovine and porcine cell types
- Support basic research to understand the Type I interferon locus in cattle and swine and how the protein products of these genes affect innate and adaptive immune responses
- Determine the differential expression of the IFNα genes in bovine and porcine
- Develop technologies for analyzing the adaptive immune response to infection and vaccination
- Determine correlates between cellular immune responses and vaccine efficacy

Vaccines

- Develop vaccinal needle-free strategies to induce mucosal as well as systemic responses in susceptible species
- Develop vaccine formulations effective in neonatal animals with or without maternal immunity
- Investigate the safety and efficacy characteristics of novel attenuated FMD vaccine platforms (e.g. leaderless FMDV)
- Understand and overcome the barrier of serotype- and subtype-specific vaccine protection (achieve cross-protection and/or increasing the breadth of antigenic coverage)
- Design and engineer second-generation immune refocused FMDV antigens
- Improve the onset and duration of immunity of current and next generation FMD vaccines
- Develop next generation FMD vaccines that prevent FMDV persistence
- Invest in the discovery of new adjutants to improve the efficacy and safety of current inactivated FMD vaccines. Current oil adjuvant formulations may have undesirable side-effects and alum-based adjutants are not effective
- Develop vaccine formulations and delivery targeting the mucosal immune responses

Biotherapeutics

- 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

Diagnostics

- Determine the link between molecular serotyping and protective immunity. FMDV serotypes include many subtypes that do not cross react and there is a need to understand the molecular basis that governs virus neutralization.
- Support the development of new technologies for pen-side testing
- Evaluate and validate commercially available pen-side tests to "fit for purpose" for surveillance, response, and recovery
- Proof-of-concept testing of herd immunity test correlated with efficacy of vaccines.
- Identify FMDV-specific non-structural protein antigenic determinants for development of DIVA diagnostic tests
- Develop serotype specific rRT-PCR assay(s)
- Development of TIGR technology for FMD serotyping/subtyping for rapid vaccine matching and monitoring variation of the virus during an outbreak of FMD
- Assess the feasibility of infrared thermography as an FMD screening tool under different environmental field conditions in healthy and diseased animal populations. Assess the potential application of this technology to aid in the identification and sampling of suspected animals for confirmatory diagnostic testing.
- Investigate the use of artificial intelligence for the development of algorithms to recognize FMD signatures in domestic animal species (cattle, pigs).
- Assess the use of air sampling technologies and validate their use for FMDV aerosol detection in open and enclosed spaces.

Disinfectants

- Development of low cost commercially available disinfectants for use in the inactivation of FMDV on contaminated surfaces found in farm settings and other susceptible environments.

Delivery Devices

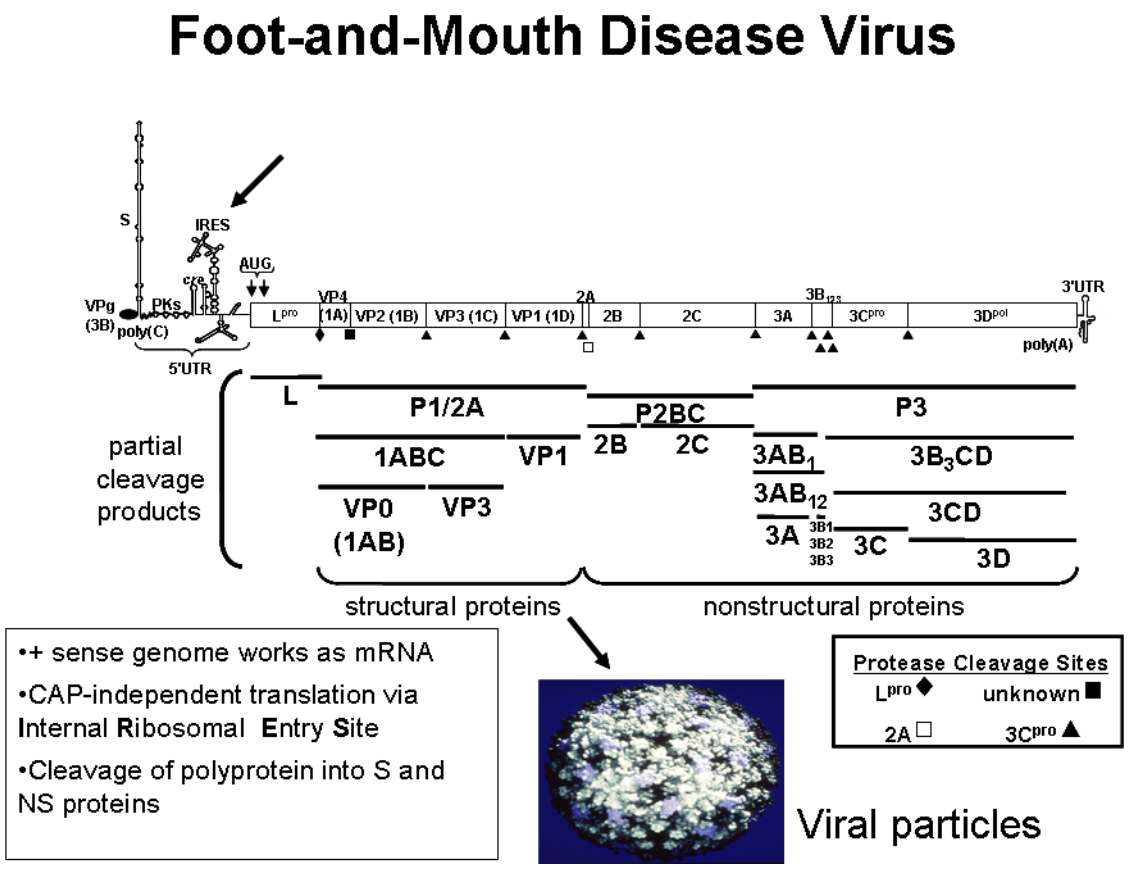
- Proof-of-concept testing of needle-free systems for the delivery of new FMD molecular vaccines and biotherapeutics.

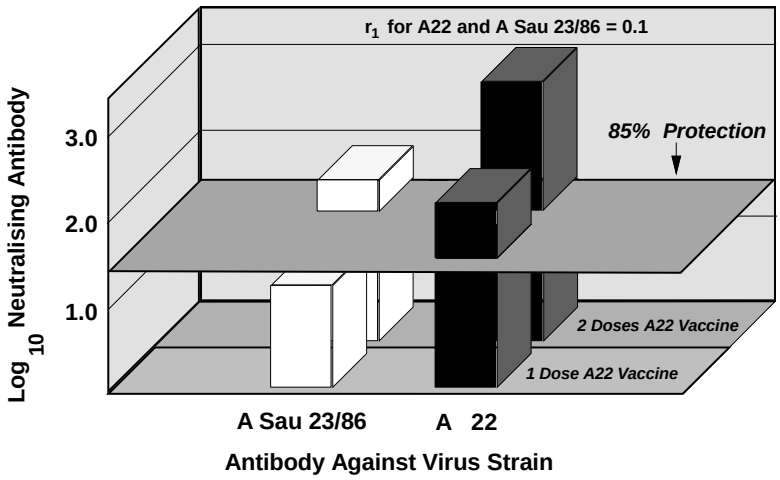
CONCLUSION

The United States is vulnerable to an accidental or intentional FMD outbreak. Seven FMDV serotypes and multiple subtypes make this disease especially difficult to control. The ecology of FMDV is poorly understood and there are no predictive tools to determine whether new strains will emerge. Depopulation still remains the primary method to eradicate FMDV in disease-free countries but the large number of livestock in the United States does not make this a viable option in the case of an epizootic. Accordingly, the FMDCWG Chair recommends stockpiling ready to use diagnostics and vaccines. Unfortunately, the very nature of this infectious disease challenges our ability to fully predict that we will have the right countermeasures in our arsenal. In addition, available countermeasures have weaknesses and there is a need for new and improved countermeasures. The FMDCWG Chair recommends improving existing countermeasures to ensure their use and integration in an eradication campaign. Priority should be given to funding research to improve diagnostics, vaccines, and biotherapeutics. Specific goals include 1) improving diagnostic tests to rapidly identify new disease strains; 2) epidemiological research to better understand virus transmission, host range specificity, and the domestic-wildlife interface; 3) develop safe and effective vaccines specifically designed for control and eradication; and 4) develop biotherapeutics or modulators of innate immunity that can significantly improve the onset of protective immunity and disease resistance.

FIGURES

Figure 1





***Source*:** C.G. Schermbrucker (unpublished results)

Figure 2
Low immunological relationship (10%) between the vaccine strain (A 22 Iraq 1964) and a field strain from Saudi Arabia (A Saudi 1986). The second injection of vaccine A 22 Iraq 1964 boosted cross-reactive neutralizing antibody levels against the A Saudi 1986 field strain above an expected protection level of 85% (white columns)

Lombard M. and A-E Fussel. *Rev. sci. tech. Off. Int. Epiz.*, 2007, 26 (1), 117-134.

Vaccinated – Protected

Unvaccinated – Unprotected

Figure 3
Digital and infrared images of vaccinated-protected and unvaccinated-unprotected cattle. Note the lower temperatures (blue-green) in the vaccinated-protected animal versus the higher temperatures (orange-red) in the unvaccinated-unprotected animal.

TABLES

Table 1: FMD diagnostic tests available now or under development

Test	What does it detect?	Development status	Capability	Utilization
FMD real time RT-PCR (rRT-PCR) Test	Virus RNA	In use	Rapid diagnostics	Primary case identification, control, and surveillance
FMD Antigen-Capture ELISA	Virus protein	In use	Routine diagnostics and confirmatory testing FADDL	Primary identification /confirmatory test
High throughput FMD rRT-PCR Test	Virus RNA	In use	Large volume sample processing on semi-automated robotic systems	Surge capability, surveillance during an outbreak, response and recovery
Multiplex FMD rRT-PCR Test	Virus RNA- rule-out vesicular look alike disease	Feasibility testing	Rapid serotype identification, rule out look alike agents	Routine surveillance
Virus isolation	Infectious virus	In use	Routine diagnostics	Confirmation
Complement fixation	Virus protein	In use	Routine diagnostics	Not in use
Solid-phase competition ELISA (SPCE)	Serotype-specific antibody	In use	Confirming previous or ongoing infection in non-vaccinated animals and for monitoring immunity conferred by vaccination	Confirmation
3ABC ELISA Serological Assay	Antibodies to non-structural proteins	Commercially available	Distinguish infected from vaccinated animals	Control and recovery phase surveillance
3D ELISA Serological Assay	Antibodies to non-structural proteins	Feasibility testing	Distinguish infected from vaccinated animals	Control and recovery phase surveillance
O1 Serological assay. SPBE	Antibodies to FMDV serotype O. Multi-species	Commercially available	Detect antibodies against FMDV Type O	Identify circulating O strain during an outbreak without vaccination
Penside antigen test	Detection of FMDV antigen in swab and tissue samples	Commercially available	Penside test for rapid assessment of FMDV antigen	Detection of viral circulation during an outbreak without vaccination

APPENDIX I

National Veterinary Stockpile
FMD Countermeasures Working Group
Instructions

Decision Model

We will use a decision model to assess potential countermeasures to stockpile. These countermeasures must significantly improve our ability to control and eradicate an outbreak of Foot-and-Mouth Disease (FMD) in the United States. The decision model is a simple tool that will allow us to focus on critical criteria for the National Veterinary Stockpile, and rank the available interventions relative to each other. The decision model is available as a Microsoft Excel spread sheet has been prepared to quantitatively assess the rankings we assign to a set of selected criteria that will lead to the selection of the highest cumulative option. We can use as many criteria as we want but the objective is to get down to the ones that will make or break success. The criteria for each intervention will be selected by the FMD Countermeasures Focus Group on April 11, 2007, but a preliminary set has been identified to expedite the process. You are encouraged to review the criteria prior to coming to the meeting and be prepared to modify the criteria as needed with the focus group on April 11, 2007. The following provides an example of criteria and assumptions for assessing vaccines.

Criteria

If a vaccine is going to be used as an emergency outbreak control tool for FMD , then we need to know: 1) is it efficacious (does it effectively eliminate virus amplification or just reduce amplification by a known log scale); 2) does it work rapidly with one dose (probably do not have time for a second dose); 3) whether it is available today from the perspective of having a reliable and rapid manufacturing process (need to know it can be up & running rapidly and will yield a predictable amount of vaccine; 4) can we get the product to the outbreak site rapidly & safely; 5) once at the site, can we get it into the target population rapidly (feedlot, cow-calf segment); 6) type of administration- mass or injected, people and equipment to do the job become important); and 7) are diagnostics available to monitor success and or DIVA compliant. While cost is important, the cost of the vaccine in an outbreak will be small in comparison to the other costs. In addition, how fast the product can be made is important because that will have a big impact on how big a stockpile will be needed. Accordingly, you will see from the Excel sheets that have been prepared for vaccines that the following critical criteria and assignment of weights for each criterion are proposed.

Weight	Critical Criteria
10	Efficacy
2	Safety
8	Available Today
10	Speed of Scale up
2	Storage
6	Distribution
8	Mass Administration
4	All Ruminants
6	DIVA Compatible
8	Dx Available
4	Cost to Implement

APPENDIX II

Commercially Available Vaccines

Assessment of Commercial Vaccines, NVS FMD CWG, August 25, 2010

Rank each Intervention (2,4,6,8, or 10) as to its importance to making a decision, only one "10" rankings a

Weight	Critical Criteria	W/O S.E. H.P	W/O/W D.E. H.P	AISA HP
10	Efficacy	8	8	4
8	Cross-within serotypes	4	4	2
6	Cross-serotype protection	0	0	0
2	> 1 duration of immunity	6	2	0
10	< week onset immunity	6	6	6
2	No maternal antibody	4	4	2
4	Two year shelf life	4	4	6
6	Safe vaccine	8	8	8
2	No high containment	0	0	0
8	DIVA compatible	6	6	6
4	Rapid scale-up	4	4	4
2	Reasonable cost	6	6	8
2	Short withdrawal	2	2	8
2	Feasibility of registration	8	8	8
6	Add new antigens	4	4	4
8	Accelarated delivery	6	6	6

Rank each Criteria 2,4,6,8 or10 on each criterion -- no more than two "10" rankings allowed

Critical Criteria	W/O S.E. H.P	W/O/W D.E. H.P	AISA HP
Efficacy	80	80	40
Cross-within serotypes	32	32	16
Cross-serotype protection	0	0	0
> 1 duration of immunity	12	4	0
< week onset immunity	60	60	60
No maternal antibody	8	8	4
Two year shelf life	16	16	24
Safe vaccine	48	48	48
No high containment	0	0	0
DIVA compatible	48	48	48
Rapid scale-up	16	16	16
Reasonable cost	12	12	16
Short withdrawal	4	4	16
Feasibility of registration	16	16	16
Add new antigens	24	24	24
Accelarated delivery	36	36	36
Value	376	368	328

APPENDIX III

Experimental Vaccines in the Pipeline

Assessment of Experimental Vaccines, NVS FMD CWG, August 25, 2010

Rank each Intervention (2,4,6,8, or 10) as to its importance to making a decision, only one "10" rankings allowed

Weight	Critical Criteria	H Ad5-Vector-Ad	FMD-LL3B3D	Dendrimeric Peptide	Stabilized VLP
10	Efficacy	4	4	2	4
8	Cross-within serotypes	2	2	2	2
6	Cross-serotype protection	0	0	0	0
6	> 1 duration of immunity	2	2	2	2
10	< week onset immunity	6	6	2	4
6	Efficacy in young animals	4	6	2	2
4	Two year shelf life	6	6	6	6
6	Safe vaccine	4	8	2	8
2	No high containment	10	4	10	10
8	DIVA compatible	8	8	8	8
8	Rapid scale-up	4	8	2	6
6	Reasonable cost	2	8	2	6
2	Short withdrawal	4	4	2	4
6	Mass vaccination	2	2	2	2
6	Add new antigens	8	8	4	6

Rank each Criteria 2,4,6,8 or10 on each criterion -- no more than two "10" rankings allowed

Critical Criteria	H Ad5-Vector-Ad	FMD-LL3B3D	Dendrimeric Peptide	Stabilized VLP
Efficacy	40	40	20	40
Cross-within serotypes	16	16	16	16
Cross-serotype protection	0	0	0	0
> 1 duration of immunity	12	12	12	12
< week onset immunity	60	60	20	40
Efficacy in young animals	24	36	12	12
Two year shelf life	24	24	24	24
Safe vaccine	24	48	12	48
No high containment	20	8	20	20
DIVA compatible	64	64	64	64
Rapid scale-up	32	64	16	48
Reasonable cost	12	48	12	36
Short withdrawal	8	8	4	8
Mass vaccination	12	12	12	12
Add new antigens	48	48	24	36
0	0	0	0	0
Value	396	488	268	416

APPENDIX IV

Vaccine Matching

Test For FMD Vaccine Matching - FMD CWG, August 24, 2010

Rank each Intervention (2,4,6,8, or 10) as to its importance to you in making a decision, no more than one "10" rankings allowed

Weight	Critical Criteria	SVNT-AL	MAbVM	AC	RCT	CFT	SPCE	LPBE	VNT
10	Validation for purpose	2	2	2	2	4	2	4	4
10	correlation to cross-protection	8	2	6	4	4	2	4	6
8	Repeatability	6	6	6	4	6	8	8	4
6	ability to detect multiple strains	8	8	8	8	8	8	8	8
6	Pan-species use	8	8	8	8	8	8	8	8
2	Cost to Implement	2	2	2	0	6	4	4	2

Rank each Criteria 2,4,6,8 or10 on each criterion -- no more than two "10" rankings allowed

Critical Criteria	SVNT-AL	MAbVM	AC	RCT	CFT	SPCE	LPBE	VNT
Validation for purpose	20	20	20	20	40	20	40	40
correlation to cross-protection	80	20	60	40	40	20	40	60
Repeatability	48	48	48	32	48	64	64	32
ability to detect multiple strains	48	48	48	48	48	48	48	48
Pan-species use	48	48	48	48	48	48	48	48
Cost to Implement	4	4	4	0	12	8	8	4
Value	248	188	228	188	236	208	248	232

commercially available (could be stock piled)
in use in reference laboratories
"pipeline" technologies under development

APPENDIX V

Detection

Diagnostics For FMD - FMD CWG, August 24, 2010

Rank each Intervention (2,4,6,8, or 10) as to its importance to making a decision, only one "10" rankings a

Weight	Critical Criteria	VI + AgELISA	Ag ELISA	LFD	LAB RT-PCR	Mobile RT-PCR	LAMP
10	Sensitivity	8	6	6	8	8	8
8	Specificity	8	8	8	8	8	8
10	Validation for purpose	10	10	4	8	2	2
8	Throughput	4	6	8	10	2	2
8	Deployable to NAHLN	0	2	8	8	10	8
8	Rapid Result	2	8	10	8	8	6
4	Viral characterisation	8	8	0	0	0	0
6	Easy to perform	2	6	8	6	8	8
2	Cost to Implement	2	2	6	2	2	8

Rank each Criteria 2,4,6,8 or10 on each criterion -- no more than two "10" rankings allowed

Critical Criteria	VI + AgELISA	Ag ELISA	LFD	LAB RT-PCR	Mobile RT-PCR	LAMP
Sensitivity	80	60	60	80	80	80
Specificity	64	64	64	64	64	64
Validation for purpose	100	100	40	80	20	20
Throughput	32	48	64	80	16	16
Deployable to NAHLN	0	16	64	64	80	64
Rapid Result	16	64	80	64	64	48
Viral characterisation	32	32	0	0	0	0
Easy to perform	12	36	48	36	48	48
Cost to Implement	4	4	12	4	4	16
Value	340	424	432	472	376	356

commercially available
in use in reference laboratories
"pipeline" technologies under development

APPENDIX VI

Freedom from Infection (with Vaccination)

Diagnostics For Freedom of FMD Infection with Vaccination - FMD CWG, August 24, 2010

Rank each Intervention (2,4,6,8, or 10) as to its importance to you in making a decision, no more than one "10" rankings allowed

Weight	Critical Criteria	EITB	NSP 3ABC ELISA	Lab RT-PCR	sLFD	IgA	3D ELISA
10	Validation to purpose	8	8	8	2	2	6
10	Specificity	8	6	10	2	6	6
8	Sensitivity	8	8	6	2	2	6
8	Throughput	2	10	8	8	4	10
6	Pan-species use	2	8	8	2	2	6
6	Deployable	2	8	8	10	8	8
4	Cost to Implement	8	4	4	4	4	4

Rank each Criteria 2,4,6,8 or10 on each criterion -- no more than two "10" rankings allowed

Critical Criteria	EITB	NSP 3ABC ELISA	Lab RT-PCR	sLFD	IgA	3D ELISA
Validation to purpose	80	80	80	20	20	60
Specificity	80	60	100	20	60	60
Sensitivity	64	64	48	16	16	48
Throughput	16	80	64	64	32	80
Pan-species use	12	48	48	12	12	36
Deployable	12	48	48	60	48	48
Cost to Implement	32	16	16	16	16	16
0	0	0	0	0	0	0
0	0	0	0	0	0	0
Value	296	396	404	208	204	348

commercially available (could be stock piled)
in use in reference laboratories
"pipeline" technologies under development

APPENDIX VII

Freedom from Infection (No Vaccination)

Diagnostics For Freedom of FMD Infection without Vaccination - FMD CWG, August 24, 2010

Rank each Intervention (2,4,6,8, or 10) as to its importance to you in making a decision, no more than one "10" rankings allowed

Weight	Critical Criteria	SPCE	VIAA	VNT	SPCE - O	LPBE	NSP 3ABC*	Lab RT-PCR	NSP 3D ELISA	sLFD	IgA
10	Validation to purpose	8	8	10	8	8	8	8	6	2	2
10	Specificity	8	8	8	8	8	8	10	8	2	6
8	Sensitivity	8	8	8	8	8	8	6	6	2	2
8	Throughput	8	2	4	8	8	10	8	10	8	4
6	Pan-species use	8	8	8	8	8	8	8	6	2	2
6	Deployable	4	6	0	10	2	8	8	8	10	8
4	Cost to Implement	4	10	2	4	6	6	4	4	4	4

Rank each Criteria 2,4,6,8 or10 on each criterion -- no more than two "10" rankings allowed

Critical Criteria	SPCE	VIAA	VNT	SPCE - O	LPBE	NSP 3ABC*	Lab RT-PCR	NSP 3D ELISA	sLFD	IgA
Validation to purpose	80	80	100	80	80	80	80	60	20	20
Specificity	80	80	80	80	80	80	100	80	20	60
Sensitivity	64	64	64	64	64	64	48	48	16	16
Throughput	64	16	32	64	64	80	64	80	64	32
Pan-species use	48	48	48	48	48	48	48	36	12	12
Deployable	24	36	0	60	12	48	48	48	60	48
Cost to Implement	16	40	8	16	24	24	16	16	16	16
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
Value	376	364	332	412	372	424	404	368	208	204

commercially available (could be stock piled)
in use in reference laboratories
"pipeline" technologies under development

APPENDIX VIII

Herd Immunity

Diagnostics For FMD Herd Immunity- USDA/ARS, August 24, 2010

Rank each Intervention (2,4,6,8, or 10) as to its importance to you in making a decision, no more than one "10" ranki

Weight	Critical Criteria	VNT	SPCE	LPBE	SDLPBE
10	Validation for purpose	2	2	6	6
10	correlation to protection	6	6	8	8
8	Throughput	2	8	8	10
4	ability to detect multiple strains	8	8	8	8
6	Pan-species use	8	8	8	8
6	Deployable	0	8	8	8
6	Cost to Implement	2	4	4	6

Rank each Criteria 2,4,6,8 or10 on each criterion -- no more than two "10" rankings allowed

Critical Criteria	VNT	SPCE	LPBE	SDLPBE
Validation for purpose	20	20	60	60
correlation to protection	60	60	80	80
Throughput	16	64	64	80
ability to detect multiple strains	32	32	32	32
Pan-species use	48	48	48	48
Deployable	0	48	48	48
Cost to Implement	12	24	24	36
Value	188	296	356	384

	commercially available (could be stock piled)
	in use in reference laboratories

APPENDIX IX

Biotherapeutics

Assessment of Biotherapeutics, NVS FMD CWG, August 25, 2010

Rank each Intervention (2,4,6,8, or 10) as to its importance to making a decision, only one "10" rankings allowed

Weight	Critical Criteria	Adeno-INF Type 1	RNAi/PMO	Receptor block	Polymerase inh.
10	Efficacy	6	2	2	4
10	Safety	6	6	6	2
6	One dose	10	6	4	2
4	Speed of Scaleup	8	8	8	8
8	Stability/Shelf Life	8	8	8	8
6	Storage/Distribution/Supply	8	8	8	8
8	Mass Administration	4	4	4	8
6	Ruminants/Pigs	2	2	2	2
6	Withdrawl	8	6	6	2
6	Cost to Implement	4	2	4	2

Rank each Criteria 2,4,6,8 or10 on each criterion -- no more than two "10" rankings allowed

Critical Criteria	Adeno-INF Type 1	RNAi/PMO	Receptor block	Polymerase inh.
Efficacy	60	20	20	40
Safety	60	60	60	20
One dose	60	36	24	12
Speed of Scaleup	32	32	32	32
Stability/Shelf Life	64	64	64	64
Storage/Distribution/Supply	48	48	48	48
Mass Administration	32	32	32	64
Ruminants/Pigs	12	12	12	12
Withdrawl	48	36	36	12
Cost to Implement	24	12	24	12
Value	440	352	352	316

APPENDIX X

TECHNICAL ADVANTAGES OF ANTIGEN BANKS

Source: Lombard M. and Füssel A-E., 2007.

1. Consistency in the manufacturing of vaccine batches. Several runs of inactivation of several thousand liters of industrial virus harvests can be pooled as raw antigens. Equally, several pools of raw antigens can be processed to obtain highly concentrated and purified batches of bulk antigens, resulting in up to seven million doses at a potency of 6 PD_{50} in a volume as small as 50 L. A concentration factor of approximately 300 is very common but not frequently exceeded due to the increased antigen losses.

2. Possibility of formulating stored antigens at several different time points, possibly years apart, into the same final vaccine preparation. The shelf-life of the final product starts from the time the vaccine is formulated without reference to the time that the antigen was produced. Today, between 90% and 95% of FMD vaccines are produced routinely by manufacturers using antigens from antigen stocks, which means that the virus production units and vaccine manufacturing units can operate independently.

3. Blends of several batches of monovalent bulk antigens can be formulated into trial vaccines and fully tested before storage. The blends can ensure that any vaccine produced from a given controlled antigen will meet the minimum requirements of the European Pharmacopoeia, or other established requirements. During the storage time, periodic tests are conducted to ensure that the antigenic characteristics (antigen content and immunogenicity) of the antigen stocks have not deteriorated.

4. Option to calibrate the final vaccine composition, which is an extension of the third advantage and is commonly used by manufacturers but rarely by bank owners. Starting from the same bulk antigen, several blends made up of different antigen payloads can be tested to adjust the composition of the final vaccine according to the protection level required by the disease situation in the field. Consequently, different compositions of the same bulk antigen can be processed to produce final vaccine preparations with an expected potency ranging from 3 to10 PD_{50}. This is a true breakthrough for manufacturers who are, therefore, not obliged to wait for the vaccine control results and can adjust the vaccine potency according to the specification required by the contracting party in response to the emergency situation and the immunological relationship of the vaccine strain to the particular field virus. Consequently, the number of doses available in the antigen bank can vary according to the antigen payload selected to produce the final vaccine preparation, and must therefore always be expressed in relation to the expected potency.

5. The rapidity with which the antigens can be turned into the final vaccine is an important issue. Because the antigens have been fully tested before storage it is technically possible to produce the final vaccine product within a few days of the receipt and registration of an official order. The possibility of the emergency release of vaccines formulated from antigen stocks without waiting for the completion of the quality controls, as permitted by the European Pharmacopoeia and the US Code of Federal Regulations, providing that the formulation unit complies with the EU GMP requirements, or in the case of the U.S, USDA regulatory requirements, is another major advantage of maintaining antigen banks. In the EU, vaccines against FMD are an exception in terms of standard authorization procedures, which have been outlined in the monograph of the European Pharmacopoeia, but not in the *Terrestrial Manual* at the present time. Practically, authorization exception for the early release of emergency vaccine is always used by a client facing an FMD crisis and this explains the very short period of time between the receipt of the order by the manufacturer and the delivery of the vaccine on site, which varies between four and thirteen days according to shipping distance and flight availability.

6. Banks that contain highly purified antigen resulting from in-depth purification of bulk antigens has demonstrated the elimination, to a very large extent, of non-structural proteins (NSPs) of the FMD virus (FAO Report, 2001). Non-structural proteins occur as a result of FMD virus replication and are considered markers of infection. However, because one copy of the NSP, called 3D or Virus Infection Associated Antigen (VIAA), remains attached to the capsid of a high proportion of virions, complete NSP elimination is not possible. Recently, serological tests have been developed to detect in a vaccinated population those animals that have been infected with replicating FMD virus. These tests rely on the detection of antibodies to the NSP of the FMD virus which are evidence of viral replication in the animal (see analysis of DIVA diagnostic tests on pages 52-53).

APPENDIX XI

TECHNICAL DISADVANTAGES OF ANTIGEN BANKS

Source: Lombard M. and Füssel A-E., 2007.

1. Difficulties in producing concentrated and purified antigens are not easily overcome since the integrity of the inactivated virus particles (the antigen) has to be maintained during the freezing stage, the storage stage, and the thawing and dilution processes required for vaccine preparation. If the total antigen losses in the final vaccine product are greater than 50% of the initial quantity of virus particles, the process loses much of its advantage and the cost per vaccine dose prepared in this way is commercially non-viable. Industrial know-how is therefore the most important factor for the manufacturer and the profitability of his operation, and for the bank owner who expects the product quality to be similar to a freshly made product. Presently, virus particle recovery, expressed in micrograms of antigen, after production of the final vaccine product is about 70%, which signifies that 30% or more of the virus particles from the initial cultures are regularly lost during the manufacturing process.

2. Antigen losses occur during storage at –130°C. At this ultra-low temperature, virus particles rupture or aggregate over time. This phenomenon is not well documented; firstly, because stability seems to be strain-dependant and secondly, because the data are proprietary and not readily published by manufacturers (Lombard M., et al., 2003). It is accepted and considered to be normal by manufacturers that 10% of the initial virus particles will be lost within the first five years of storage of highly purified antigens. A very limited number of studies have demonstrated that after 14 years of storage up to 40% of the antigen mass may be lost. Such data clearly indicates that the storage duration for strategic reserves is limited and do not support a 'buy and store indefinitely' policy. Regular monitoring and quality control are necessary during the storage period.

3. The list of antigens stored in the bank may not contain the appropriate antigens to respond to a particular epidemiological need. Like several other animal pathogens, FMDV has a range of diverse serotypes and a large number of strains within some of the serotypes to which there is limited cross-immunity. Consequently, there is a probability that the list of antigens retained in an antigen bank may not match or provide immunity against a new pathogen appearing in the field and may become obsolete over a ten year storage period depending on how much the epidemiological situation has changed. For example, in 1996 a severe A22 related virus outbreak was observed in Albania. The only suitable type A antigen available in the EU FMD antigen bank at the time of the outbreak was the A22 Iraq 1964 virus, which was ranked with a serological relationship of only 30% (r1=0.3) with the newly emerged virus. Despite the low serological relationship, a joint decision was made by the EU Commission and the EU FMD antigen bank to use the A22 Iraq vaccine against the A22 Albania-96 virus and to inject two doses at one month intervals to achieve the level of immunity necessary to stop the epizootic. A similar observation related to a Saudi outbreak is illustrated in Figure 2. As demonstrated recently by the UK FMD outbreak in 2001, viruses occurring in any region of the world are a

potential threat to all other regions, no matter how far away, and consequently should also be considered for inclusion in national or regional antigen banks. Strain selection is a complex responsibility for manufacturers and bank owners. An antigen collection should strive to reflect the major strains involved in recent epidemiological situations and also the strains expected to be involved in potential epidemiological situations in the next five years. However, this attempt is often hampered because the standard sera produced by manufacturers from their vaccines are considered proprietary and prevents governments or international organizations from being able to constantly match the existing antigens against an evolving epidemiological situation.

4. Even when properly stored and monitored carefully by owners or manufacturers, antigen strategic reserves are vulnerable to terrorism, accidents, or other unpredictable destructive events. Strategic reserves are valuable assets and essential materials for governments and international organizations. Consequently, security should be guaranteed in all cases. One of the solutions to minimizing risks associated with strategic reserves involves splitting the antigen reserves between two or more storage sites that are situated at a considerable distance from one another (Fussel A-E., 2004). Having more than one storage and adjacent formulation facility is also very convenient when different orders requesting different emergency vaccines are submitted at the same time.

APPENDIX XII
MANUFACTURERS AND DISTRIBUTORS

I. VACCINE MANUFACTURERS, with regulatory approval for one or more vaccines

A. FMD vaccine manufacturers, sorted by country providing regulatory approval:

Afghanistan

Manufacturer: Jordanian Vaccine Company (JOVAC)

Product	**Vaccine Type**	**Strain**	**Adjuvant**
AFTOVAC (trivalent)	Killed Vaccine	O1, A22, Asia1	Not Available
AFTOVAC (monovalent)	Killed Vaccine	O1	Not Available
AFTOVAC (Bivalent)	Killed Vaccine	O1, A22	Not Available

Albania

Manufacturer: Jordanian Vaccine Company (JOVAC)

Product	Vaccine Type	Strain	Adjuvant
AFTOVAC (trivalent)	Killed Vaccine	O1, A22, Asia1	Not Available
AFTOVAC (monovalent)	Killed Vaccine	O1	Not Available
AFTOVAC (Bivalent)	Killed Vaccine	O1, A22	Not Available

Argentina

Manufacturer: Biogénesis S.A.

Product	Vaccine Type	Strain	Adjuvant
Product Name Not Available	Killed Vaccine	South American, O97-Taiwan, formulations vary	Oil and Saponin
Product Name Not Available	Killed Vaccine	A24-Cruzeiro, A79-Argentina, A87/A81-Argentina, A2000-Argentina, A2001-Argentina, O1-Campos, O1-Caseros, O97-Taiwan, C3-Indaial, C3 85-Argentina	Oil and Saponin

Bahrain

Manufacturer: Jordanian Vaccine Company (JOVAC)

Product	Vaccine Type	Strain	Adjuvant
AFTOVAC (trivalent)	Killed Vaccine	O1, A22, Asia1	Not Available
AFTOVAC (monovalent)	Killed Vaccine	O1	Not Available
AFTOVAC (Bivalent)	Killed Vaccine	O1, A22	Not Available

Botswana

Manufacturer: Botswana Vaccine Institute

Product	Vaccine Type	Strain	Adjuvant
Product Name Not Available	Killed Vaccine	Types O, A, SAT1, SAT2, SAT3	Aluminum hydroxide and saponin

Brazil

Manufacturer: Akzo Nobel Ltda.

Product	Vaccine Type	Strain	Adjuvant
Product Name Not Available	Killed Vaccine	O1-Campos, A24-Cruzeiro, C3-Indaial	Oil

Manufacturer: Bayer S.A.

Product	Vaccine Type	Strain	Adjuvant
Product Name Not Available	Killed Vaccine	O1-Campos, A24-Cruzeiro, C3-Indaial	Oil
Product Name Not Available	Killed Vaccine	O1-Campos, A24-Cruzeiro	Oil

Manufacturer: Coopers Brasil Ltda.

Product	Vaccine Type	Strain	Adjuvant
Product Name Not Available	Killed Vaccine	O1-Campos, A24-Cruzeiro, C3-Indaial	Oil

Manufacturer: Merial Saúde Animal

Product	Vaccine Type	Strain	Adjuvant
Product Name Not Available	Killed Vaccine	O1-Campos, A24-Cruzeiro, C3-Indaial	Oil
Product Name Not Available	Killed Vaccine	O1-Campos, A24-Cruzeiro	Oil

Manufacturer: Vallée SA

Product	Vaccine Type	Strain	Adjuvant
Product Name Not Available	Killed Vaccine	O1-Campos, A24-Cruzeiro,	Oil

Colombia

Manufacturer: Empresa Colombiana de Productos Veterinarios S.A. (Vecol)

Product	Vaccine Type	Strain	Adjuvant
Product Name Not Available	Killed Vaccine	A24-Cruzeiro, O1-Campos	Not Available

Manufacturer: Laboratorios Laverlam S.A.

Product	Vaccine Type	Strain	Adjuvant
Product Name Not Available	Killed Vaccine	A24-Cruzeiro, O1-Campos	Oil

Manufacturer: Limor de Colombia

Product	Vaccine Type	Strain	Adjuvant
Product Name Not Available	Killed Vaccine	O1-Campos, A24-Cruzeiro	Oil

Egypt

Manufacturer: Veterinary Serum and Vaccine Research Institute

Product	Vaccine Type	Strain	Adjuvant
Product Name Not Available	Killed Vaccine	Serotype O	Aluminum hydroxide
Product Name Not Available	Killed Vaccine	O1-Minisa	Aluminum hydroxide

Ethiopia

Manufacturer: Jordanian Vaccine Company (JOVAC)

Product	Vaccine Type	Strain	Adjuvant
AFTOVAC (trivalent)	Killed Vaccine	O1, A22, Asia1	Not Available
AFTOVAC (monovalent)	Killed Vaccine	O1	Not

AFTOVAC (Bivalent)	Killed Vaccine	O1, A22	Available Not Available

France

Manufacturer: Merial SAS

Product	Vaccine Type	Strain	Adjuvant
Product Name Not Available	Killed Vaccine	Not available	Oil

Germany

Manufacturer: Intervet International GmbH

Product	Vaccine Type	Strain	Adjuvant
Product Name Not Available	Killed Vaccine	Serotypes O, A, Asia, SAT1, SAT2, SAT3	Yes

India

Manufacturer: Indian Immunologicals Limited

Product	Vaccine Type	Strain	Adjuvant
Product Name Not Available	Killed Vaccine	Types O, A, C, Asia-1	Oil
Product Name Not Available	Killed Vaccine	Types O, C, Asia-1, A22	Aluminum hydroxide and saponin

Manufacturer: Intervet India

Product	Vaccine Type	Strain	Adjuvant
Product Name Not Available	Killed Vaccine	Types O, A, Asia-1, C	Yes

Iran

Manufacturer: Razi Vaccine and Serum Research Institute

Product	Vaccine Type	Strain	Adjuvant
Product Name Not Available	Killed Vaccine	O, A, and Asia-1	Saponin and Aluminum hydroxide

Iraq

Manufacturer: Jordanian Vaccine Company (JOVAC)

Product	Vaccine Type	Strain	Adjuvant
AFTOVAC (Bivalent)	Killed Vaccine	O1, A22	Not Available
AFTOVAC (trivalent)	Killed Vaccine	O1, A22, Asia1	Not Available
AFTOVAC (monovalent)	Killed Vaccine	O1	Not Available

Jordan

Manufacturer: Jordanian Vaccine Company (JOVAC)

Product	Vaccine Type	Strain	Adjuvant
AFTOVAC (Bivalent)	Killed Vaccine	O1, A22	Not Available
AFTOVAC (trivalent)	Killed Vaccine	O1, A22, Asia1	Not Available
AFTOVAC (monovalent)	Killed Vaccine	O1	Not Available

Kenya

Manufacturer: KARI Veterinary Vaccines Production Centre

Product	Vaccine Type	Strain	Adjuvant

Product Name Not Available	Not Available	A,O, SAT1, and SAT2	Not Available

Kuwait

Manufacturer: Jordanian Vaccine Company (JOVAC)

Product	Vaccine Type	Strain	Adjuvant
AFTOVAC (Bivalent)	Killed Vaccine	O1, A22	Not Available
AFTOVAC (trivalent)	Killed Vaccine	O1, A22, Asia1	Not Available
AFTOVAC (monovalent)	Killed Vaccine	O1	Not Available

Lebanon

Manufacturer: Jordanian Vaccine Company (JOVAC)

Product	Vaccine Type	Strain	Adjuvant
AFTOVAC (Bivalent)	Killed Vaccine	O1, A22	Not Available
AFTOVAC (trivalent)	Killed Vaccine	O1, A22, Asia1	Not Available
AFTOVAC (monovalent)	Killed Vaccine	O1	Not Available

Libya

Manufacturer: Jordanian Vaccine Company (JOVAC)

Product	Vaccine Type	Strain	Adjuvant
AFTOVAC (Bivalent)	Killed Vaccine	O1, A22	Not Available
AFTOVAC (monovalent)	Killed Vaccine	O1	Not Available
AFTOVAC (trivalent)	Killed Vaccine	O1, A22, Asia1	Not Available

Malaysia

Manufacturer: Jordanian Vaccine Company (JOVAC)

Product	Vaccine Type	Strain	Adjuvant
AFTOVAC (Bivalent)	Killed Vaccine	O1, A22	Not Available
AFTOVAC (monovalent)	Killed Vaccine	O1	Not Available
AFTOVAC (trivalent)	Killed Vaccine	O1, A22, Asia1	Not Available

Netherlands

Manufacturer: ID-LELYSTAD

Product	Vaccine Type	Strain	Adjuvant
Product Name Not Available	Killed Vaccine	A22-Irak, A24-Cruzeiro, A-Tur 14/98, Asau 23/86, Asau 41/91, A5-Westerwald,C1-Detmold, Asia-1 Shamir, O1-Manisa, O1-BFS, O-Taiwan, O-Algeria	Aluminum hydroxide and saponin
Product Name Not Available	Killed Vaccine	A22-Irak, A24-Cruzeiro, A-Tur 14/98, Asau 23/86, Asau 41/91, A5-Westerwald, C1-Detmold, Asia-1 Shamir, O1-Manisa, O1-BFS, O-Taiwan, O-Algeria	Oil

Manufacturer: Intervet International B.V.

Product	Vaccine Type	Strain	Adjuvant
Product Name Not Available	Killed Vaccine	O, A, C, Asia 1, SAT1, SAT2	Oil or Aluminum hydroxide and saponin

Oman

Manufacturer: Jordanian Vaccine Company (JOVAC)

Product	Vaccine Type	Strain	Adjuvant
AFTOVAC (Bivalent)	Killed Vaccine	O1, A22	Not Available
AFTOVAC (monovalent)	Killed Vaccine	O1	Not Available
AFTOVAC (trivalent)	Killed Vaccine	O1, A22, Asia1	Not Available

Pakistan

Manufacturer: Jordanian Vaccine Company (JOVAC)

Product	Vaccine Type	Strain	Adjuvant
AFTOVAC (Bivalent)	Killed Vaccine	O1, A22	Not Available
AFTOVAC (monovalent)	Killed Vaccine	O1	Not Available
AFTOVAC (trivalent)	Killed Vaccine	O1, A22, Asia1	Not Available

Paraguay

Manufacturer: Lauda Sociedad Anónima Paraguaya

Product	Vaccine Type	Strain	Adjuvant
Product Name Not Available	Killed Vaccine	Serotypes O, A, C	Not available

Russia

Manufacturer: Agrovet

Product	Vaccine Type	Strain	Adjuvant
Product Name Not Available	Killed Vaccine	A22, O1	Not Available
Product Name Not Available	Killed Vaccine	Type A	Not Available
Product Name Not Available	Killed Vaccine	Asia-1	Not Available
Product Name Not Available	Killed Vaccine	Type O	Not Available
Product Name Not Available	Killed Vaccine	Type C	Not Available
Product Name Not Available	Killed Vaccine	Types O, A, Asia-1	Not Available
Product Name Not Available	Killed Vaccine	Types O, A, C	Not Available

South Africa

Manufacturer: Onderstepoort Biological Products

Product	Vaccine Type	Strain	Adjuvant
Product Name Not Available	Killed Vaccine	SAT1, SAT2, SAT3	Aluminum hydroxide

Syria

Manufacturer: Jordanian Vaccine Company (JOVAC)

Product	Vaccine Type	Strain	Adjuvant
AFTOVAC (Bivalent)	Killed Vaccine	O1, A22	Not Available
AFTOVAC (monovalent)	Killed Vaccine	O1	Not Available
AFTOVAC (trivalent)	Killed Vaccine	O1, A22, Asia1	Not Available

Thailand

Manufacturer: Bureau of Veterinary Biologics

Product	Vaccine Type	Strain	Adjuvant
Product Name Not Available	Killed Vaccine	Serotypes O, A, Asia I	Aluminum gel
Product Name Not Available	Killed Vaccine	Serotypes O, A, Asia I	Oil

Manufacturer: FMD Center

Product	Vaccine Type	Strain	Adjuvant
Product Name Not Available	Killed Vaccine	Serotypes O, A, Asia 1	Aluminum
Product Name Not Available	Killed Vaccine	Serotypes O, A, Asia 1	Oil

Turkey

Manufacturer: Vetal Company

Product	Vaccine Type	Strain	Adjuvant
Product Name Not Available	Killed Vaccine	O1, A22, Asia-1	Single water/oil emulsion

United Arab Emirates

Manufacturer: Jordanian Vaccine Company (JOVAC)

Product	Vaccine Type	Strain	Adjuvant
AFTOVAC (Bivalent)	Killed Vaccine	O1, A22	Not Available
AFTOVAC (monovalent)	Killed Vaccine	O1	Not Available
AFTOVAC (trivalent)	Killed Vaccine	O1, A22, Asia1	Not Available

United Kingdom

Manufacturer: Merial Animal Health Ltd.

Product	Vaccine Type	Strain	Adjuvant
Product Name Not Available	Killed Vaccine	Not available	Aluminum hydroxide and saponin
Product Name Not Available	Killed Vaccine	Not available	Oil

Venezuela

Manufacturer: C.A. Laboratorios Asociados (CALA)

Product	Vaccine Type	Strain	Adjuvant
Product Name Not Available	Killed Vaccine	O1, A24	Oil
Product Name Not Available	Killed Vaccine	O1, A24	Oil

Yemen

Manufacturer: Jordanian Vaccine Company (JOVAC)

Product	Vaccine Type	Strain	Adjuvant
AFTOVAC (Bivalent)	Killed Vaccine	O1, A22	Not Available
AFTOVAC (monovalent)	Killed Vaccine	O1	Not Available
AFTOVAC (trivalent)	Killed Vaccine	O1, A22, Asia1	Not Available

B. FMD vaccine manufacturers, alphabetic list:

AGROVET (RUSSIA)
Address: 23 Academic Skryabin Street, 109472 Moscow, Russia
Phone: 7.495.377.69.97; **Fax:** 7.495.377.69.87
Email: info@agrovet.ru
Website: http://www.agrovet.ru/index.eng.htm

AKZO NOBEL LTDA. (BRAZIL)
Address: Rua Prof. Vicente Silveria, 234 - Fortaleza - CF Brasil, Brazil
Phone: 55.85.256.9200; **Fax:** 55.85.256.3437
Email: info.br@intervet.com

BAYER S.A. (BRAZIL)
Address: Rua Edú Chaves, 360 Porto Alegre - R.S. Brasil, Brazil
Phone: 55.51.3342.2777; **Fax:** 55.51.3342.2287

BIOGÉNESIS S.A. (ARGENTINA)
Address: Ruta Panamericana Km 38,5 (B1619IEA). Garín Provincia de Buenos Aires, Argentina
Phone: 54-3327-448362; **Fax:** 54-3327-448347
Email: info@biogenesisbago.com
Website: http://www.biogenesisbago.com

BOTSWANA VACCINE INSTITUTE
Address: Plots 6385/90, Lejara Road Broadhurst Industrial Estate, Private Bag 0031 Gaborone, Botswana
Phone: 267.391.2711; **Fax:** 267.395.6798
Email: gmatlho@bvi.co.bw
Website: http://www.bvi.co.bw

BUREAU OF VETERINARY BIOLOGICS (THAILAND)
Address: Department of Livestock Development, 1213 Pak Chong, Nakornratchasima 30130, Thailand
Phone: 66.44.311.476; **Fax:** 66.44.315.931
Email: gbiologic@dld.go.th

C.A. LABORATORIOS ASOCIADOS (CALA; VELEZUELA)
Address: Venezuela

COOPERS BRASIL LTDA.
Address: Av. Sir Henry Wellcome, 336 - Cotia – SP, Brazil
Phone: 55.11.4612.2495

EMPRESA COLOMBIANA DE PRODUCTOS VETERINARIOS S.A. (VECOL; COLUMBIA)
Address: Av. El Dorado #82-93, Bogotá, Colombia
Phone: 263.3100; **Fax:** 263.8331

FMD CENTER (THAILAND)
Address: Pakchong Nakornratchasima, Thailand 30130, Thailand
Phone: 66.44.311.592; **Fax:** 66.44.312.870
Email: wilaifmd@loxinfo.co.th

ID-LELYSTAD (WAGENINGEN UNIVERSITY AND RESEARCH CENTRE, THE NETHERLANDS)
Address: PO Box 65, Edelhertweg 15, 8200 AB Lelystad, The Netherlands
Phone: 31.320.293262; **Fax:** 31.320.238961
Email: remco.schrijver@wur.nl
Website: http://www.asg.wur.nl

INDIAN IMMUNOLOGICALS LIMITED
Address: Road # 44, Jubilee Hills, Hyderabad 500033, A.P., India
Phone: 267.391.2711; **Fax:** 267.395.6798
Email: info@indimmune.com
Website: http://www.indimmune.com

INTERVET (INDIA)
Address: 33, Nagar Road, Pune 411014, India
Phone: 31.485.585228; **Fax:** 31.485.587419
Email: info@intervet.com
Website: http://www.intervet.com

INTERVET INTERNATIONAL B.V. (THE NETHERLANDS)
Address: Wim de Körverstraat 35, PO Box 35, The Netherlands
Phone: 31.485.587600; **Fax:** 31.485.587491
Email: info@intervet.com
Website: http://www.intervet.com

INTERVET INTERNATIONAL GMBH (GERMANY)
Address: Betriebsstatte Köln, Osterather Str. 1a, 50739 Köln, Germany
Phone: 31.485.587600; **Fax:** 31.485.587491
Email: info@intervet.com
Website: http://www.intervet.com

JORDANIAN VACCINE COMPANY (JOVAC)
Address: Jordan Bio-Industries Center, Marketing Dept, PO Box 43, Jordan
Phone: 962.6.5602451; **Fax:** 962.6.5602451
Email: amjad_jovac@yahoo.com

KARI VETERINARY VACCINES PRODUCTION CENTRE (KENYA)
Address: PO Box 57811, City Square. Nairobi, 00200, Kenya
Phone: 254.020.4183720 ; **Fax:** 254.020.4183344
Email: resource.center@kari.org
Website: http://www.kari.org/kevevapi/vaccines.htm

LABORATORIOS LAVERLAM S.A. (COLUMBIA)
Address: Cra. 42B #22C-49, Bogotá, Colombia
Phone: 244.3039; **Fax:** 447.4009
Email: laver-bo@bogota.cetcol.net.co

LAUDA SOCIEDAD ANÓNIMA PARAGUAYA
Address: Inglaterra y Capitán Grau 2.909, Asunción, Paraguay
Phone: 595.21.290.776; **Fax:** 595.21.291.498

LIMOR DE COLOMBIA
Address: Av. 15 #106-50 PH2, Bogatá, Colombia
Phone: 529.9397; **Fax:** 529.9415

MERIAL ANIMAL HEALTH LTD. (UNITED KINGDOM, PIRBRIGHT)
Address: Biological Laboratory, Ash Rd, Pirbright, Surrey GU24 ONQ, United Kingdom
Phone: 00.44.1483.235.331; **Fax:** 00.44.1483.235.330
Website: http://uk.merial.com/

MERIAL SAÚDE ANIMAL (BRAZIL)
Address: Av. Carlos Grimaldi, 1701 4 andar, CEP 13091-908, Campinas, Brazil
Phone: 55.19.3707.5022; **Fax:** 55.19.3707.5101
Email: emilio.salani@merial.com
Website: http://www.merial.com

MERIAL SAS (FRANCE)
Address: 29, avenue Tony Garnier 69007, LYON cédex 07, France
Phone: 04.72.72.30.00; **Fax:** 04.72.72.30.69
Website: http://www.merial.com/index.asp

ONDERSTEPOORT BIOLOGICAL PRODUCTS (SOUTH AFRICA)
Address: Private Bag X05, Onderstepoort, 0110, South Africa
Phone: 2712.529.9111; **Fax:** 2712.529.9595
Email: Wilna@Saturn.ovi.ac.za
Website: http://www.up.ac.za/academic/veterinary

RAZI VACCINE AND SERUM RESEARCH INSTITUTE (IRAN)
Address: PO Box 31975/148, Post No. 3197619751, Karaj, Iran
Phone: 98.261.4570038/46; **Fax:** 98.261.4552194
Email: Razi_Institute@rvsri.com
Website: http://www.rvsri.com

VALLÉE SA (BRAZIL)
Address: Av. Hum, 1500, Montes Claros - MG Brasil, Brazil
Phone: 55.38.3229.7000 ; **Fax:** 55.38.3229.7000
Website: http://www.vallee.com.br

VETAL COMPANY (TURKEY)
Address: Gölbasi Yolu Üzeri 7 Km Adiyaman, Turkey
Phone: 90.416.223.2030; **Fax:** 90.416.223.1456
Email: vetal@vetal.com.tr
Website: http://www.vetal.com.tr/indexeng.htm

VETERINARY SERUM AND VACCINE RESEARCH INSTITUTE (EGYPT)
Address: Cairo, Abbasia, El-Sekka El-Beida St. Egypt, PO Box 131, Post Code 11381, Egypt
Phone: 202.38224406; **Fax:** 202.6858321
Email: svri@idsc.gov.eg

II. VACCINE MANUFACTURERS, without regulatory approval

Bharat Biotech/Biovet (India)
Biovet is a private venture by the founder of Bharat Biotech, Dr. Krishna Ella.

Bharat Biotech is primary manufacturer of vaccines and therapeutics for humans and has been in operations since 1998. BBIL facilities are located in Hyderabad India.

The veterinary vaccines will be manufactured at the facilities of Biovet in Bangalore India. Biovet is developing a one of a kind BSL3+ manufacturing facility in India to manufacture FMD vaccines. The facilities will be completed shortly and manufacturing would commence during Q4 2007. We are already working with Indian regulatory agencies for the licensure of this vaccine, which should be completed 3-6 months after the completion of the facility. This will be a dedicated facility for FMD vaccines for veterinary use. It may also be used for other vaccines that require such containment facilities. Excerpted from email to Elsken from: prasadsd@bharatbiotech.com.

GenVec http://www.genvec.com/

GenVec, Inc. (NASDAQ: GNVC) is a biopharmaceutical company developing novel gene-based therapeutic drugs and vaccines, using GenVec's proprietary adenovector technology to develop vaccines for infectious diseases.

GenVec has capitalized on expertise with their platform adenovector technology and accompanying 293-ORF6 cell line used to produce the needed adenovectors to form funded collaborations for the development of vaccine candidates. Current collaborations include the Vaccine Research Center/National Institute of Allergy and Infectious Diseases/National Institutes of Health for the development of both HIV, Influenza (seasonal and pandemic) and RSV vaccine candidates, Naval Medical Research Center and Malaria Vaccine Initiative for the development of malaria vaccine candidates, and the United States Department of Agriculture/Agricultural Research Services and the Department of Homeland Security for the development of anti-virals and vaccines to treat foot and mouth disease in large hoofed animals.

GenVec Inc., 65 West Watkins Mill Road, Gaithersburg, MD 20878
Telephone: (240) 632-0740 or (877) 943-6832
Fax: (240) 632-0735

III. COMMERCIAL DIAGNOSTIC TEST KIT MANUFACTURERS

ANIGEN ANIMAL GENETICS, INC.
Address: 404-5Woncheon-dong Yeongtong-gu, Suwon-si, Kyunggi-do, Korea
TEL:82-31-211-0516, 0968 FAX:82-31-211-0537
Website: http://www.anigen.co.kr

GREENSPRING FMD IGG DISTINGUISHING ELISA KIT
SHENZHEN LVSHIYUAN BIOTECHNOLOGY CO., LTD.
Address: Rm. 507, No.2., Longgang Overseas Venture Park, Shenzhen, Guangdong, China

IDEXX Europe B.V. (*idexx HAS purchased Bomelli.com FMD Chekit kit*)
Address: Koolhovenlaan 20, 1119 NE Schiphol-Rijk, The Netherlands
Tel: 31-20-655-23-00; Fax: 31-20-655-23-33
Website: http://www.idexx.com/production/index.jsp

SVANOVA BIOTECH AB
ADDRESS:
VISITOR ADDRESS: EDELHERTWEG 15, 8219 PH LELYSTAD, THE NETHERLANDS
TEL: +31 320 238320; FAX: +31 320 214379
WEBSITE: HTTP://WWW.SVANOVA.COM/

See: http://www.fao.org/ag/againfo/commissions/docs/research_group/greece04/App66.pdf
Serial release testing for FMD ELISA kits: necessity of official control
Karen Luyten, Nesya Goris1, Ann-Brigitte Caij1, Kris De Clercq1*
**,1Veterinary Agrochemical Research Centre, Groeselenberg 99, 1180 Ukkel, Belgium*

IV. VACCINATION EQUIPMENT MANUFACTURERS

A. Needle-free vaccine delivery devices currently on the market:

- Agro-Jet

http://www.mitcanada.ca/products/products.html
MEDICAL INTERNATIONAL TECHNOLOGIES (MIT CANADA) INC.
1872 Rue Beaulac
Ville St-Laurent
Montreal, Quebec
Canada H4R 2E7
Tel: 514.339.9355
Fax: 514.339.2885
E-mail: marketing@mitcanada.ca

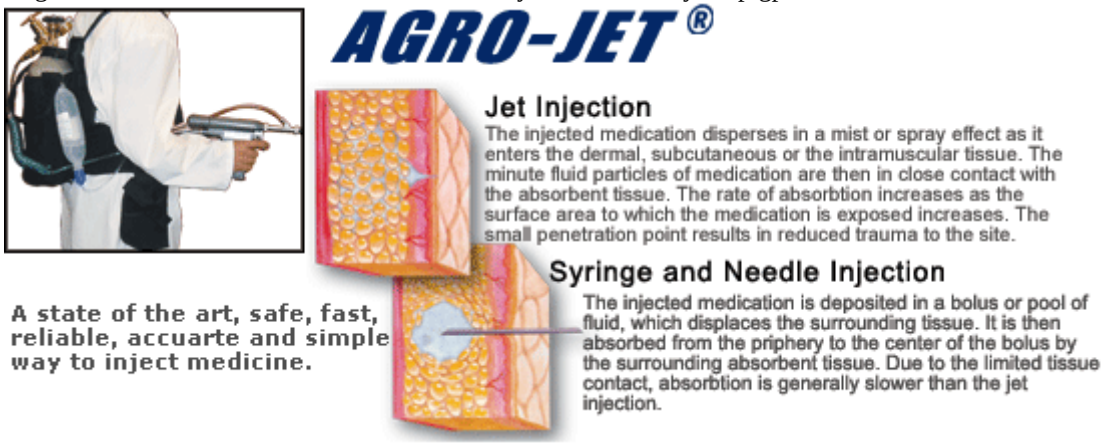
Manufacturers' information:
The **AGRO-JET®** is a semi-automatic, high performance, needle free jet injector specifically designed for the livestock, poultry and small & companion animal industries. The AGRO-JET® can deliver medications of from 0.1 to 5cc at speeds of 600 to 3,000 injections an hour - a much higher amount than other existing needle-free injectors. The system's fine adjustability allows extremely precise dosage delivery, while the high absorption rate can result in a medication savings of up to 15 percent.

The difference between MIT's AGRO-JET® needle free jet injection system from other systems is its ability to utilize low pressure delivery methods without compromising accuracy, convenience and ease of use - while ensuring patient comfort, environmental safety and user affordability.

- Less pain and stress
- Reduces tissue damage
- Provides high absorption rate
- No biological waste
- Highly cost effective
- Significant time savings
- No cross contamination
- Allows intradermal, subcutaneous or intra-muscular injections

The **AGRO-JET®** offers a range of features:

- Automatic, semi-automatic or manual operation
- Powered by CO2 (gas pressure) or compressed air, most ideal for situations calling for mass or multiple injections in animals.
- The CO2 canister is conveniently carried on the back in a specially designed harness bag, freeing the hands and allowing for free movements and can be carried everywhere around your pigpen or farm.



- Bioject DERMA-VAC NF (Needle Free Transdermal Vaccination System)

http://www.bioject.com/

Mailing Address, Phone, and Fax:

Bioject Medical Technologies, Inc.
20245 S.W. 95th Avenue
Tualatin, Oregon 97062
Phone:503. 692.8001
Fax: 503.692.6698

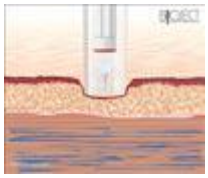
Manufacturers' information:

Technology Overview

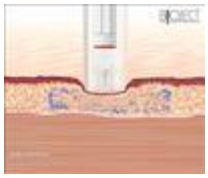
Needle-free injection - What is it?

Bioject's needle-free injection technology works by forcing liquid medication at high speed through a tiny orifice that is held against the skin. The diameter of the orifice is smaller than the diameter of a human hair. This creates an ultra-fine stream of high-pressure fluid that penetrates the skin without using a needle.

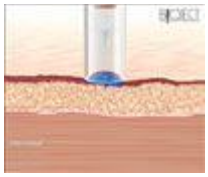
Bioject's technology is unique because it delivers injections to a number of injection depths and supports a wide range of injection volumes. For instance, the Biojector 2000 can deliver intramuscular or subcutaneous injections up to 1 mL in volume. In addition, Bioject is developing a syringe for the Biojector 2000 that delivers intradermal injections that is currently in clinical trials.



Intramuscular injections are the deepest injection type, delivering the medication into the muscle tissue. Most vaccines are currently delivered to the intramuscular depth.



Subcutaneous injections are delivered to the adipose (fat) layer just below the skin. Many therapeutic proteins are delivered to the subcutaneous depth, such as human growth hormone.



Intradermal* injections are very shallow injections that deposit the medication between the layers of the skin. Many new DNA-based vaccines are delivered to the intradermal layer. * In Clinical Trials

- IDAL® Vaccinator (Intervet),

http://www.thepigsite.com/focus/intervet/3425/idal-vaccinator-needleless-intradermal-application-of-vaccines

Mailing Address:
Intervet, Inc.
P.O. Box 318
29160 Intervet Lane
Millsboro, DE 19966

Manufacturers' information:
IDAL Vaccinator is a revolutionary needleless vaccination device, that allows intradermal application of swine vaccines.

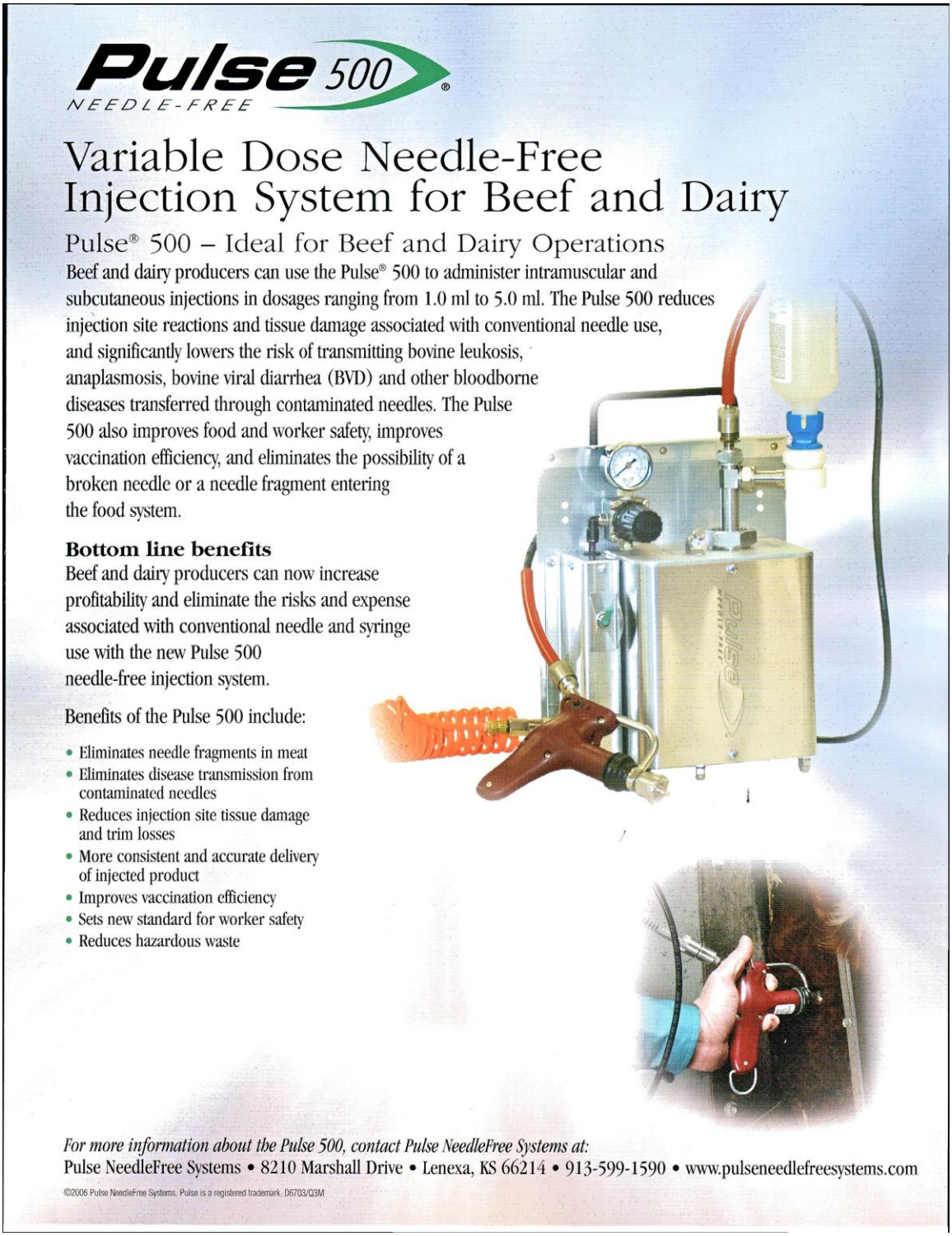
Due to its unique nature it has a number of advantages over traditional vaccination techniques.

Advantages of intradermal application

- It is postulated that an intra-dermal application is making a better use of the dendritic cells which will give stronger cellular immunity. A recent Italian trial that used peripheral lymphocyte-subpopulations (CD4 and CD8) as a parameter showed a difference in favor of ID application (Borghetti, 2003).
- Intra-dermal application is with 0.2 ml Diluvac Forte. This contains the same antigen dose as a regular intra-muscular dose, the concentration is thus 10 times higher.
- IDAL applies without a needle and therefore has not the risk of PRRS-virus transmission via the needle as described between others by Otake (Otake, 2001).
- IDAL circumvents the risk of any abscesses in muscle or sub-cutaneous tissue. Especially in small piglets the animal-friendly application is appreciated, both by the piglets and the operators.

- PulseTM Micro Dose Injection System (Pulse Needle Free Systems),

http://www.pulse-nfs.com/products/



Pulse 500®
NEEDLE-FREE

Variable Dose Needle-Free Injection System for Beef and Dairy

Pulse® 500 – Ideal for Beef and Dairy Operations

Beef and dairy producers can use the Pulse® 500 to administer intramuscular and subcutaneous injections in dosages ranging from 1.0 ml to 5.0 ml. The Pulse 500 reduces injection site reactions and tissue damage associated with conventional needle use, and significantly lowers the risk of transmitting bovine leukosis, anaplasmosis, bovine viral diarrhea (BVD) and other bloodborne diseases transferred through contaminated needles. The Pulse 500 also improves food and worker safety, improves vaccination efficiency, and eliminates the possibility of a broken needle or a needle fragment entering the food system.

Bottom line benefits

Beef and dairy producers can now increase profitability and eliminate the risks and expense associated with conventional needle and syringe use with the new Pulse 500 needle-free injection system.

Benefits of the Pulse 500 include:

- Eliminates needle fragments in meat
- Eliminates disease transmission from contaminated needles
- Reduces injection site tissue damage and trim losses
- More consistent and accurate delivery of injected product
- Improves vaccination efficiency
- Sets new standard for worker safety
- Reduces hazardous waste

For more information about the Pulse 500, contact Pulse NeedleFree Systems at:
Pulse NeedleFree Systems • 8210 Marshall Drive • Lenexa, KS 66214 • 913-599-1590 • www.pulseneedlefreesystems.com

©2006 Pulse NeedleFree Systems. Pulse is a registered trademark. D6703/Q3M

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Alexandersen, S., Quan, M., Murphy, C., Knight, J., Zhang, Z., 2003. Studies of quantitative parameters of virus excretion and transmission in pigs and cattle experimentally infected with foot-and-mouth disease virus. J.Comp Pathol. 129, 268-282.

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