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

One of the key products of the GFRA is to conduct in-depth gap analyses to assess advances made in the scientific discovery and development of veterinary medical countermeasures to effectively support the global control and eradication of FMD. These gap analyses are conducted periodically through the organization of workshops with the aim of updating the GFRA Gap Analysis Report. The objective is to ensure the research conducted by GFRA members is relevant and addresses the priorities most likely to have the greatest impact in the control of FMD.

The latest workshop to update the GFRA Gap Analysis Report was organized in Buenos Aires June 2018 with the support of the United States Department of Agriculture (USDA) and the Instituto Nacional de Tecnología Agropecuaria (INTA).

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GFRA Gap Analysis Working Group - June 14, 2018
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
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 FMD outbreaks, and also support global control and eradication initiatives in FMD-endemic countries. The working group was organized with the support of the Global Foot-and-Mouth Disease Research Alliance (GFRA), the United States Department of Agriculture (USDA), and the Instituto Nacional de Tecnología Agropecuaria (INTA). The GFRA Gap Analysis Working Group met in Buenos Aires, Argentina, June 14-17, 2018.

Gap Analysis

The GFRA Gap Analysis Working Group identified several remaining obstacles to effectively prevent, detect, and control FMD, including:

1. Lack of validated commercial pen-side test kits for disease control.
2. Failure of serologic methods to determine status (infected, uninfected) in some vaccinated animals.
3. Absence of a surveillance system for early recognition of signs, or to find evidence using antigen detection, antibody, or virus detection.
4. Lack of reliable comprehensive international surveillance systems to collect and analyze information.
5. Current models have not been designed to evaluate in real-time the cost-effectiveness of alternative control, surveillance, and sampling strategies.
6. 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.
7. 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.”
8. There is a need for better analytical tools to support decisions for FMD control.
The GFRA Gap Analysis Working Group 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 the engineering of next generation FMD vaccines that can be safely manufactured.

**Diagnostics**

Several commercial serologic (antibody-based) diagnostic test kits, including DIVA test kits have been developed and are available. Validated real-time (r)RT-PCR assays have been developed for use in reference laboratories; 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 available and currently being evaluated and validated for use. All tests should be evaluated for compliance with World Organisation for Animal Health (OIE) 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 should be (further) developed, including:

- anomaly detection methods to identify outlier events
- 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
- 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
- enhanced application of molecular epidemiological approaches to tracing of movement of established and emergent FMDV strains
- development and standardized of tools to enable utilization of NGS-derived subconsensus sequence data for enhanced tracing
- continued investigation of the relevance of subclinically infected animals in the propagation of contagion, including carriers and acute (neoteric) subclinical infections.

**Viral Pathogenesis**

- Continued investigation of determinants of virulence for different serotypes and strains of FMDV in cattle, sheep, pigs, Asian buffalo, and African buffalo.
- Continued investigation of virus-host interactions at the primary sites of infection in ruminants and pigs with focus on factors defining tropism, generalization, and early host responses.
- Elucidate viral and host mechanisms of FMDV persistence in ruminants with goal of identifying mechanisms which may be subverted through vaccines, countermeasures, or post-exposure therapy
- Determine characteristics and mechanisms of FMDV within-host evolution over distinct phases of infection
- Gain understanding of species-specific and breed-specific continuum of permissiveness/tolerance/resistance to clinical and sub-clinical infection
- Improved understanding of onset and duration of infectiousness from clinically and sub-clinically infected animals
- Elucidate viral and/or host mechanistic determinants of highly successful emergent lineages (PanAsia, Ind2001a-e)

**Immunology**

- Elucidate mucosal responses to acute and persistent infections in cattle
- Establish the immune mechanisms underlying protection against FMDV throughout distinct phases 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, $\gamma$δ T cells, and DCs)
• Contract the development of antibodies to surface markers of critical immune bovine and porcine cell types as well as specific for bovine IFN$\alpha$ and $\beta$ as well as porcine IFN$\beta$
• 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 IFN$\alpha$ genes in bovine and porcine tissues
• 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 adjuvants 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 hours) 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 for the purpose of control and eradication, FMD must be considered as 7 distinct diseases. FMDV is transmitted by direct or indirect contact through infected hosts or 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.

FMDV is highly contagious to bovidae and suidae, including cattle, sheep, goats, pigs, as well as many wild ruminants and suids. The morbidity of FMD is high; however, the disease is rarely fatal in adult animals. Under specific virus-host relationships high mortality may occur 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 shed by infected animals before clinical signs are evident. Characteristic clinical signs of FMD include vesicles (blisters) and erosions on the mouth, tongue, lips, feet and udder. Ancillary clinical signs include ptyalism (excessive salivation), fever, lameness, weight-loss and reduced milk production. However, there is substantial variation in severity of the clinical syndrome depending both on the host species, immune status (innate and vaccinal), and intrinsic properties of the virus strain. Upon recovery from FMD, approximately 50% of ruminants become ‘carriers’ with persistent subclinical infection of the upper respiratory tract. The epidemiological importance of FMDV carriers remains incompletely elucidated, but is generally believed to be low. However, these animals are critically important for FMD outbreak control in countries that are normally free of FMD as the presence of such animals drives outbreak response policies. Additionally, the existence of the FMDV carrier state, and the (perceived) risk of carriers amongst vaccinated livestock has profoundly impacted the regulation of international trade in animal products as well as the waiting times required to prove freedom of FMD when vaccination is used to control FMD outbreaks. Importantly, currently available FMD vaccines do not prevent subclinical or persistent FMDV infection. FMDV vaccines prevent clinical disease, but not infection with FMDV.

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 United Nation Food Agricultural Organization (FAO) 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, the GFRA working group was charged with the task of conducting an in-depth analysis of available countermeasures to control and eradicate FMD. This report provides the results of this analysis. The GFRA working group 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 working group 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

GFRA Gap Analysis Workshops (2010 and 2018)

This gap analysis report is the compilation of assessments conducted during scientific conferences organized by the GFRA 2008-2018 and two comprehensive gap analyses conducted during two workshops organized by the GFRA Executive Committee in 2010 and 2018. These two GFRA Gap Analysis Workshops included FMD experts from government research institutions, diagnostic laboratories, academia, and industry. The 1st GFRA Gap Analysis Workshop was organized with the support of the United States Department of Agriculture (USDA), Agricultural Research Service (ARS) and the Instituto Nacional de Tecnologia Agropecuaria (INTA), and GFRA Partners, Collaborators, and Stakeholders. The workshop took place at INTA Headquatters, Buenos Ares, Argentina, August 24-25, 2010. The 2nd GFRA Gap Analysis Workshop was also organized with the support of USDA-ARS and INTA, and GFRA Partners, Collaborators, and Stakeholders. The workshop took place at INTA Headquarter, Buenos Ares, Argentina, June 12-14, 2018. Instructions (see Appendix I) and several reference materials were provided by the GFRA working group Chair prior to the meeting. The GFRA Gap Analysis Working Groups were tasked by the Chair with assessing the best available countermeasures to rapidly and effectively control and eradicate an FMD outbreak. When gaps in the available information necessary to complete the analysis were identified, members of the working group contacted additional experts as needed (see list of contributors on Page 108).

Report Updates

This report will be updated periodically with new scientific information, research breakthroughs, and/or information on the successful development of veterinary medical countermeasures. This report was last updated with the support of GFRA December 2018.

Expert Reports

The GFRA Working Groups 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


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

GFRA Published Reviews:


CURRENT GAPS AND VULNERABILITIES

The following section summarizes the status of our understanding of FMD virology, pathogenesis, immunology, epidemiology, and the available tools to effectively detect, control and eradicate FMDV, including a summary of the FMD situation worldwide and current obstacles for controlling FMD.

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 (Borio, Inglesby et al. 2002, Sidwell and Smee 2003). FMD is widely recognized as one of the greatest threats and constraints to global trade in livestock and animal-derived products. The highly contagious nature of FMDV and the associated productivity losses make it a primary animal health concern worldwide.

VIROLOGY
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. Field strains evolve rapidly which requires vigilance for efficacy of existing vaccine strains for contemporary strains. 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, O'Neill et al. 2007, Brito, Pauszek et al. 2018).

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 Lpro, 2A, 2B, 2C, 3A, 3B1-3, 3Cpro, and 3Dpol. Lpro, 3Cpro, and 2A are proteases that mediate cleavage of the viral polyprotein and, in the case of Lpro and 3Cpro, they also cleave specific host proteins (Belsham, McInerney et al., 2000;Birtle & 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 αvβ1, αvβ3, αvβ6 and αvβ8) 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)(Lawrence, Pacheco et al. 2016)(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 Lpro 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 cytolitic 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. Theses 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. Primary 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 via the oral route 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 infection via the respiratory tract (Alexandersen & Donaldson, 2002; Stenfeldt, Pacheco et al. 2014). Asymptomatic 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 oropharyngeal 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 remain poorly defined (Sutmoller and Casas 2002, Tenzin, Dekker et al. 2008, Garland and de Clercq 2011, Bertram, Vu et al. 2018).

**PATHOGENESIS**

Recent work has elucidated many aspects of virus-host interactions; yet important gaps remain. Enduring gaps in our understanding of the molecular events of early pathogenesis still limit the design and development of completely effective countermeasures which may induce sterile immunity. It remains clear that rapid systemic dissemination with high titer viral replication and dysregulated host immune responses are central elements of viral success at the individual host level.

Understanding of primary infection processes in different species is critical to development of next generation countermeasures which may ultimately prevent infection, not just prevent clinical disease. Numerous studies have now demonstrated that in cattle, primary infection occurs in specialized regions...
the nasopharyngeal mucosa (Burrows, Mann et al. 1981, Brown, Piccone et al. 1996, Arzt, Pacheco et al. 2010, Stenfeldt, Eschbaumer et al. 2015, Stenfeldt et al., unpublished). The first cells to become infected are cytokeratin-containing epithelial cells overlaying regions of the mucosa-associated lymphoid tissue (MALT). These cells slough, leaving areas of erosion, from which the virus moves into the underlying lymphoid tissue. It is suspected that viremia is established in this process; however some studies have suggested that viremia may be established in the lungs. Recent work using contact exposure system has suggested that infection of the lungs may not be a critical step in establishment of viremia. (Stenfeldt et al., unpublished). Primary infection is quite similar in pigs, except the critical regions of primary infection are in the oropharyngeal tonsils (para-epiglottic tonsil and palatine tonsil) rather than the nasopharynx (Stenfeldt, Pacheco et al. 2014). Despite the distinct anatomic site, the morphologic and mechanistic features are exquisitely similar to cattle. Sheep seem to share some attributes of primary infection of both cattle and pigs (Stenfeldt, Pacheco et al. 2015); however, less work has been done in small ruminants. Overall, this improved understanding of primary sites of infection should be viewed as a potential target to exploit with novel countermeasures. Specifically, enhancement of mucosal immunity is likely to produce a substantially improved prophylactic effect.

In pigs, primary FMDV infection has been localized to epithelial crypts of the oropharyngeal tonsils (Stenfeldt, Pacheco et al. 2014). Additionally, these same anatomic sites support substantial FMDV replication during the clinical phase of disease, as demonstrated by the occurrence of characteristic micro-vesicular lesions within the tonsillar crypt epithelium. In both cattle and pigs, primary infection is followed by establishment of viremia, coincidently with further viral amplification at peripheral lesion (vesicle) predilection sites. In the context of countermeasures development, it should be noted that it is critical that prophylactic products target these pre-viremic events in the upper respiratory or upper gastrointestinal tracts. Thus, enhancement of mucosal immunity has high probability of improving protection. 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 5 days after viremia is first detected, followed by the appearance of circulating antibodies (Stenfeldt, Heegaard et al. 2011, Eschbaumer, Stenfeldt et al. 2016). Elimination of virus at secondary sites of infection usually takes 10 to 14 days (Oliver, Donaldson et al., 1988; Oliver, Donaldson et al., 1988). Pigs are efficient in complete clearance of infectious FMDV within 28 days after infection (Stenfeldt, Pacheco et al. 2016). However, in domestic and wild ruminants, FMDV may persist (i.e. carrier state) with intermittent viral shedding in the oropharyngeal 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 in epithelium of the nasopharyngeal MALT, or associated lymphoid tissue (Juleff et al., 2008; Stenfeldt, Eschbaumer et al.
Substantial effort has been invested in recent years to elucidate viral and host mechanisms of establishment and maintenance of persistence (Parthiban, Mahapatra et al. 2015, Eschbaumer, Stenfeldt et al. 2016, Maree, de Klerk-Lorist et al. 2016, Stenfeldt, Eschbaumer et al. 2016, Stenfeldt, Eschbaumer et al. 2017). Complete elucidation remains elusive, but much has been learned.

It is particularly noteworthy that both primary and persistent FMDV infection in cattle have been associated with the same regions of epithelium of the nasopharyngeal mucosa (Arzt, Pacheco et al. 2010, Stenfeldt, Eschbaumer et al. 2015, Stenfeldt, Eschbaumer et al. 2016), suggesting unique virus-host relationship at that site. Recent investigations based on transcriptomic analyses of nasopharyngeal tissues from FMDV carriers and animals that had cleared infection suggests that FMDV persistence is associated with an impaired cellular immune response and inhibition of apoptotic pathways (Eschbaumer, Stenfeldt et al. 2016, Stenfeldt, Eschbaumer et al. 2017).

The true role of FMDV carriers 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 (Hedger and Condy 1985). Additionally, recent experimental studies have confirmed that intranasopharyngeal inoculation of naïve cattle with oropharyngeal fluid from persistently infected cattle leads to fulminant FMD, despite the presence of secreted antibody (Arzt, Belsham et al. 2018). 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 $10^3$ 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). Recent investigations have also reported of relative lymphopenia during early FMDV infection of cattle (Perez-Martin, Weiss et al. 2012, Eschbaumer, Stenfeldt et al. 2016). 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 FMDV infection 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 have been 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). Additionally, recent investigations have demonstrated differences in gene expression at the site of FMDV persistence between FMDV carriers and animals that successfully cleared infection. Specifically, the transitional phase of infection, which corresponds to the temporal window during which FMDV is cleared from the nasopharynx of cattle that do not become carriers, was associated with an enhanced cellular immune response in animals that cleared infection (Stenfeldt, Eschbaumer et al. 2017).

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 (Lpro), 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,
Further, the IFN-γ 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). In contrast to pigs, multiple investigations have demonstrated a strong systemic type I/III IFN response in cattle, coinciding with establishment of viremia (Reid, Juleff et al. 2011, Stenfeldt, Heegaard et al. 2011, Perez-Martin, Weiss et al. 2012, Eschbaumer, Stenfeldt et al. 2016).

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).

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. found that both bovine IgG1 and IgG2 antibody-antigen complexes are able to fix bovine complement in vitro but IgG1 might be more efficient than IgG2 (McGuire, Musoke et al. 1979). 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, Arzt, Pacheco et al. 2014, Stenfeldt, Arzt et al. 2018), 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, Stenfeldt, Eschbaumer et al. 2016), transmission of FMD by contact exposure to FMDV carrier cattle 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 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.

Update on the immunity against FMDV in susceptible species

<table>
<thead>
<tr>
<th>Gap identified</th>
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<tr>
<td>Determine the role of cellular innate immune responses in FMDV infection of cattle and swine</td>
<td>ongoing</td>
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<tr>
<td>Determine the differential expression of the IFNα genes in bovine and porcine</td>
<td>ongoing</td>
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<tr>
<td>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</td>
<td>ongoing?</td>
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<tr>
<td>Develop methods to activate cells of the innate response to anti-viral activity (NK cells, γδ T cells, and DCs)</td>
<td>ongoing?</td>
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<tr>
<td>Contract the development of antibodies to surface markers of</td>
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Innate immunity

A large number of *in vitro* studies have shown that pathogen-associated molecular patterns (PAMP) within the FMDV structure may be promptly recognized by different host cells capable of activating early anti-viral responses, mainly mediated by type I and type III interferons (IFNs). Both cytokines form part of the early innate immune responses and also represent a link between immune cells from the innate and adaptive immune systems (Fensterl et al., 2015).

The study of the mechanisms behind the innate immunity against FMDV in animals has been closely related to fundamental works describing the *in vitro* interaction of viral proteins with host proteins and cellular structures to circumvent a variety of anti-viral mechanisms triggered upon infection. During the last ten years, several reports have demonstrated in different susceptible cell types that both non-structural (Lpro, 2B, 2C, 3A, and 3Cpro) (Zhu et al., 2016, Wang et al., 2011, Gladue et al., 2012, de los Santos et al., 2009, Gladue et al., 2014) and structural proteins (VP1, VP2, and VP3) (Li et al., 2016, Sun et al., 2018), as well as non-coding regions of the genome (3’ and 5’UTR) (Rodriguez-Pulido et al., 2011, Kloc et al., 2017) have been shown to affect, directly or indirectly, the occurrence of anti-viral responses driven by type I/III IFNs. Such interactions occur through different pathways and may affect transcription, translation, cellular trafficking and secretion of different effector and intermediary proteins, as well as promote membrane rearrangements and cellular autophagy. However, their actual occurrence in infected animals and their impact in preventing protection against the infection have not been clearly demonstrated *in vivo* so far.

Numerous *in vivo* experiments have demonstrated the local and systemic induction of type I/III IFNs concurrently with the onset of the viremic phase after infection in naïve cattle (Stenfeldt et al., 2011, Windsor et al., 2011, Arzt et al., 2014). Such induction requires the presence of active FMDV; therefore, these innate responses do not occur in cattle immunized with conventional inactivated vaccine.
formulations. Similarly, good quality vaccines, which impedes generalization of the infection as well as the development of the viremia in the infected animals, also prevents systemic IFN production after experimental challenge (Eschbaumer et al., 2016). As a whole, this evidence would also indicate that the natural induction of anti-viral IFNs in cattle triggered by the infectious virus, does not prevent significantly the progression of the infection.

Systemic production of IFN has been associated with blood-derived and tissue-resident plasmacytoid dendritic cells (pDC) in cattle. These cells are a major source of type I IFN in different species but ex vivo assays have shown that only live FMDV particles complexed with antibodies are able to stimulate pDC to produce this cytokine (Reid et al., 2011). Such timing of type I IFN induction, after the development of neutralizing antibodies, would suggest that this cytokine does not play a major role in controlling infection, at least from this cellular source.

Other authors have shown that PBMC taken from cattle during the viremic phase of the infection (24h-48h post-infection) exhibited an increased production of mRNA for IFNβ, IFNλ3, and for an array of IFN-stimulated genes (ISG) (Perez-Martin et al., 2012). Similarly, other reports have also identified an increased activity of type I/III IFNs soon after infection but at secondary replication sites and in the presence of large quantities of virus and viral RNA (Arzt et al., 2014, Zhang et al., 2009). These findings would suggest that non-lymphoid cells at distal sites may also function as potential sources of the systemic IFNs. Interestingly, innate responses at primary infection sites are less evident, with much lower magnitudes than for the systemic detection and contrasting observations regarding activation and inhibition of innate immune processes (Stenfeldt et al., 2018). This would probably indicate a direct correlation between the extent of the FMDV replication and the activation of the innate immune responses.

Reports on the interplay of FMDV with bovine immune cells also differ respect the relevance of this interaction. Unlike swine, most of the literature indicate that FMDV infection does not produce significant decrease in lymphocyte populations or compromise the immunocompetence in cattle (Windsor et al., 2011). Further reports, however, have shown that live FMDV associated to specific antibodies as immune-complexes (IC) may infect and kill bovine monocyte-derived DC (moDC) in vitro, altering its natural tropism toward integrin-bearing epithelial cells (Robinson et al., 2011). Other publications indicated that both the number of circulating DC and the expression of MHC-II molecules may be significantly reduced, but during the peak of viremia and in the absence of antigen-specific antibodies. According to these authors, this interaction during the acute phase of the infection, would impair the ability to process exogenous antigens by different DC subsets (Sei et al., 2016).

Early reports have also demonstrated that ex vivo infection of porcine circulating pDC with FMDV IC results in the production of type I IFN (Guzyllack-Piriou et al., 2006). In contrast to cattle, however, evidence of the in vivo production of type I IFN in swine is still controversial. Some authors have detected IFNα in serum concomitant with the onset of viremia with magnitudes depending on the infecting serotype (Nfon et al., 2010). Interestingly, this report also indicates a significant depletion of pDCs in peripheral blood during FMDV infection, thus suggesting alternative sources for these responses (Summerfield, 2012). Contrarily, other studies found that there was no detectable induction of systemic IFNα in naïve pigs following challenge with FMDV (Diaz-San Segundo et al., 2010). It is interesting to note, though, that exogenous administration of both IFNs, alone or combined with type II (γ) IFN, has shown its efficacy as antiviral agents in swine (Moraes et al., 2007, Dias et al.,
2011, Perez-Martin et al., 2014). Animals treated with type I IFN one day prior FMDV infection, had increased numbers of dendritic cells (skin) and natural killer cells (lymph nodes), as well as increased levels of several ISGs at 1 and 2 dpi (Diaz-San Segundo et al., 2010). These results may denote that FMDV would have certainly evolved molecular pathways to interfere with the endogenous production of systemic IFN in the swine, although further studies are required to assess such hypothesis. In any case and as it was mentioned for bovines, the accumulated evidence seems to indicate that the natural induction of anti-viral IFNs subsequent to the infection has a limited effect in preventing generalization of the infection within the animals.

Adaptive immunity

The induction of FMDV-specific immunity in the infected or vaccinated animals has been typically characterized by the study of humoral responses detected in serum samples (Morgan et al., 1970). An abundant number of reports indicate that serum antibody titers, measured by classical serological assays such virus-neutralization tests (VNT) and liquid-phase blocking ELISA (LPBE), keep a significant correlation with protection against challenge with a homologous virus strain in target species (Maradei et al., 2008, Chen et al., 2007). In recent years, additional immune parameters and mechanisms were also considered, seeking for more comprehensive approaches to explain the immune processes underlying protection.

The development of new protocols enabling FMDV aerogenous infection in bovines by controlled aerosol exposure (Pacheco et al., 2010), also allowed the study of the induction of the adaptive immunity along the respiratory tract, the main entry portal of the virus in the field. The study of the time-course of FMDV-specific antibody-secreting cells (ASC) induced in lymphoid organs following aerogenous challenge, established for the first time that cattle effectively develop a rapid and vigorous genuine local antibody response throughout the respiratory tract (Pega et al., 2013). The early induction, starting at 4 dpi, as well as the progress of the Ig isotype profiles indicated the development of a T cell-independent antibody response which drove the IgM-mediated virus clearance in cattle infected by FMDV aerosol exposure. These findings were in line with previous reports indicating the T-cell independent nature of the anti-FMDV antibody responses induced after infection in cattle (Juleff et al., 2009) and laboratory animal models (Borca et al., 1986).

Following this same strategy, these authors also studied the generation of FMDV-specific ASC along the respiratory tract after parenteral FMD vaccination and subsequent aerogenous infection of the vaccinated animals (Pega et al., 2015). FMDV-specific ASC, predominantly IgM, were detected from 7 dpv to 29 dpv in lymph nodes all along the whole respiratory tract and distant from the vaccination site. Oronasal infection with the homologous virus strain of these animals resulted in complete protection and triggered a local anamnestic response upon contact with the replicating FMDV, suggesting that FMD vaccination also induces the circulation of virus-specific B lymphocytes, including memory B cells that differentiate into ASC soon after contact with the infective virus.

The actual ability of virus-specific antibodies in preventing the development of FMD in cattle infected by the aerogenous route was studied in animals passively immunized with homologous immune sera from FMD-vaccinated bovines extracted at different times post-vaccination (7 and 26 dpv) (Barrionuevo et al., 2018). This report demonstrates that circulating antibodies, in the absence of other active immune mechanisms, may prevent generalization of the infection when present in sufficient titers. Interestingly, animals vaccinated and challenged with the homologous strain only 7 days after vaccination, developed a potent booster response soon after challenge, characterized by a rapid rise in total and neutralizing
antibody titers, a clear change in the isotype profile and a fast increase in the avidity of the immune serum, which were detected at both systemic and mucosal levels.

The requirement of the presence of antigen-specific CD4(+) T-cell responses for the induction of FMDV-specific humoral responses was also analyzed in vaccinated cattle (Carr et al., 2013). Contrarily to the results obtained for the immune response developed after infection in naïve cattle (Juleff et al., 2009), virus neutralizing antibody titers in cattle vaccinated with an inactivated FMD commercial formulation were significantly reduced and class switching delayed following in vivo CD4(+) T-cell depletion. Moreover, PBMC cultures from these vaccinated animals in vitro stimulated with inactivated FMDV antigens were found to induce antigen-specific CD4(+) T-cell proliferative and IFN-γ production responses. However, neither the magnitude of T-cell proliferative responses nor the extent of the IFN-γ production showed a clear correlation with the antibody responses.

The induction of FMDV-specific cellular immunity was further analyzed in vaccinated bovines. In this study, homologous and heterologous responses were evaluated in groups of animals immunized with monovalent or tetravalent FMD vaccine formulations comprising O1/Campos, A24/Cruzeiro, A/Arg/2001 and/or C3/Indaial strains (Bucafusco et al., 2015). Unlike antibody responses, FMDV-specific cell-mediated responses measured by in vitro IFN-γ production demonstrated extensive intra- and inter-serotypic cross-reactivity in whole blood samples from FMD-vaccinated cattle. Furthermore, viral strains differed in their ability to elicit FMDV-specific IFN-γ responses, both in vivo and ex-vivo, in close relation to the stability of their corresponding whole capsid particles (140S).

Early reports investigated on the relevance of the antibody affinity in terms of the protection against FMDV challenge in natural hosts (Steward et al., 1991) and laboratory animals (Mulcahy et al., 1992). The concept, however, was left aside until it was once again analyzed in the context of the protection against FMDV infection with heterologous strains in cattle (Lavoria et al., 2012). These authors developed an ELISA test to measure the avidity of immune serum samples taken before experimental challenge and found a direct correlation between the avidity indexes and the protection status against a heterologous strain within serotype A. These findings were further confirmed with different vaccination protocols also tested for heterologous challenge (Di Giacomo S., 2016) as well as with vaccinated cattle infected with a homologous virus strain (Barrionuevo et al., 2018).

A recent report analyzed the immunological interactions existing in sheep before and after FMD vaccination using different experimental adjuvants. In this report, authors used a systems-biology approach applied for measuring a broad swath of immunological functions as a means of discovering novel relationships between sets of cells and other interacting components of the immune system, related with the induction of FMDV-specific antibody responses. Analysis of ovine blood transcriptional modules (BTM) revealed that early after vaccination, BTM relating to myeloid cells, innate immune responses, dendritic cells, and antigen presentation correlated positively with antibody responses, whereas BTM relating to T and natural killer cells, as well as cell cycle correlated negatively (Braun et al., 2018). Such systems approaches to studying vaccination responses may have the potential to reveal ways for improving vaccines through the stimulation of parts of the immune system that are not being stimulated by the current vaccines.

During the last years a number of immunological processes, elicited after immunization with conventional inactivated FMD vaccines, have been identified. Most of them, however, were involved in
the protection against viral strains which are homologous (or with close antigenic relation) to those in the formulations. This knowledge has led to increased efficacy of the commercial vaccines utilized in the current FMD control programs.

The FMDV antigenic diversity, nevertheless, still represents a serious impediment to the deployment of successful vaccination programs in the field. The development of novel viral antigens, formulations or vaccination protocols that may induce broader antigenic responses currently signifies a challenge, as well as the understanding of the immune processes underlying the protection against heterologous FMDV strains.

High payload vaccines have demonstrated to allow cross-protection against strains within the same serotype (Nagendra Kumar et al., 2011, Brehm et al., 2008). Similarly, polyvalent formulations and multiple vaccination schedules (Duque et al., 2016, Maradei et al., 2013) have proved to enhance the cross-protective responses. However, the immunological bases of such findings have not been explained so far.

Alternative approaches are currently being explored by different research groups. These efforts cover a large range of strategies. Some of them working on the design recombinant viral antigens with enhanced antigenic breadth through the development of chimeric FMDV particles comprising a mosaic of antigenic determinants from different strains. This hypothesis though still needs to be experimentally proved in animals. Others are studying the problem from the host perspective, analyzing the antibody diversity and the immunoglobulin repertoire elicited after immunization in susceptible species. Such projects aim to isolate cross-reactive antibodies as well as to identify cross-neutralizing epitopes within the virus capsid through the generation of virus escape mutants.

Previously, FMDV-specific B-cell responses, measured as circulating ASC, were studied in cattle vaccinated using monovalent formulations (O1 Manisa or O SKR) and challenged with the O SKR strain (Grant et al., 2016). Both vaccines were able to induce cross-reactive plasma-cell responses and both groups of animals were protected against the experimental infection with the virulent O SKR strain. However, circulating FMDV O-serotype-specific memory B-cells were not detected after vaccination and further challenge. These findings are in line with the previous report by Pega et al (2015), where only low numbers of FMD-specific memory B-cells could be detected in the blood circulation from revaccinated animals soon after revaccination.

Further studies have shown the generation of cross-reactive serological response after sequential vaccination with inactivated FMD vaccines formulated with at least three different serotypes (Grant et al., 2017). These authors suggest that the ASCs generated after FMDV vaccination are likely to be short-lived extrafollicular plasma cells which remain at the site of induction, reaching the circulation only in low numbers and for a short time.

As a whole, the experimental evidence reveals the existence of potential approaches to increase the protection range, especially for strains within the same serotype. However, the immunological explanations for these observations remain unclear and certainly deserves further attention.

**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 (Knight-Jones et al., 2000).
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 and Baxt 2004, Arzt, Baxt et al. 2011, Weaver, Domenech et al. 2013). 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 and Hughes 2002, Gulbahar, Davis et al. 2007, Stenfeldt, Pacheco et al. 2014). Clinical signs of the disease are fever, 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. Similarly vaccinated animals become asymptotically infected (Stenfeldt, Eschbaumer et al. 2016, Farooq, Ahmed et al. 2018); yet transmission cycles amongst infected vaccinated animals are minimally investigated (Eble, de Koeijer et al. 2008). 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 to 15 weeks (Cottral 1969). Transmission from asymptomatic carriers is generally believed to be insubstantial (Tenzin, Dekker et al. 2008, Bertram, Vu et al. 2018); yet, recent laboratory studies have demonstrated that risk from carriers is not completely negligible (Arzt, Belsham et al. 2018).

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 novel strains have spread to regions of the world distant from the sites of origin. The best documented recent example is the spread of the serotype O, Ind2001 lineage beyond India to regions of Southeast Asia and the Middle East (Vu, Long et al. 2017, Qiu, Abila et al. 2018). The precise 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 (Parthiban, Mahapatra et al. 2015, Brito, Pauszek et al. 2017, Bertram, Vu et al. 2018, Farooq, Ahmed et al. 2018). 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, the body of literature describing FMD epidemiology continues to grow; however comprehensive understanding of the mechanisms which determine the emergence, persistence, competition, and spread of new variants of FMD viruses remains elusive.

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.
COUNTERMEASURES ASSESSMENT

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 broad reactive vaccine strains with adequate potency for effective immunological prophylaxis. All ungulates are susceptible requiring animal species-specific control strategies. Because trade restrictions impact the use 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 when a previously FMD-free region faces an outbreak.

ASSUMPTIONS

The following captures assumptions made by the GFRA Working Groups in assessing potential countermeasures to enhance our ability to contain and eradicate an outbreak of FMD in an FMD-free country. The United States was selected as a prototype country for the purpose of this analysis.

Situation
Countermeasures assessed for worst case scenario: an act of agroterrorism with two different FMDV serotypes released simultaneously on multiple sites in the United States, 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 tests are available but all test samples would be sent to national reference laboratory at the Plum Island Animal Disease Center (FADDL), NVSL, and National Animal Health Laboratory Network (NAHLN) FMD-certified laboratories for confirmatory testing.

**DECISION MODEL**
The GFRA Working Group 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 gap analysis workshop (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 GFRA Working Group selected core criteria to enable the comparison of countermeasures using a pertinent and valid analysis. For example, the following criteria were selected for ideal FMD vaccine profile:

**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 GFRA Working Group for each of the interventions reflect the collective best judgment of working group members (see Appendices II, III, IV, and V)

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 Waldmann 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 (Waldmann 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 Waldmann’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 Waldmann 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).

Aluminium hydroxide/saponin aqueous adjuvant proved to be efficacious to control and eradicate FMD in Chile (1981) and Europe (1991) (Sutmoller et al., 2003). Oil-adjuvant vaccines are usually formulated as water-in-oil single emulsion (W/O) using mineral oils, and emulsifiers (Aucouturier et al., 2001). Some manufacturers add saponin as immunomodulator (Mattion et al., 1998). These vaccines were a key factor in achieving FMD free areas in South America, a region with one of the highest cattle populations in the world as well as in other regions where pigs numbers are significant (Chen et al., 1999). Compared with aqueous vaccines, W/O vaccines have important advantages such as longer
duration of immunity, requiring less frequent revaccinations, and effectiveness in inducing protective responses not only in ruminants, but also in pigs (Augé de Mello and Gomes, 1977; Hunter, 1996; Rivenson et al., 1982b; Sutmoller et al., 2003). Double oil emulsion vaccines (water-in-oil-in water emulsion) have similar attributes as W/O vaccines (Selman et al., 2006). However, some reports documented that W/O vaccines induced higher antibody responses, and longer duration of immunity in cattle and pigs (Smitsaart et al., 2004). Both double oil emulsion and W/O vaccines prepared with newly developed adjuvants result in products of low viscosity and with appropriate efficacy, safety and stability (Aucouturier et al., 2001).

New adjuvants and immunomodulators that enhance efficacy, short and/or long-term immunity, and safety are being investigated in animal models and in target species both with inactivated whole antigen and with antigen subunits (Borrego et al., 2013; Cao, 2014; Dar et al., 2013; Quattrocchi et al., 2014; Saravanan et al., 2015). It should be noted that when assessing the performance of adjuvants in clinical trials, care should be taken when comparing studies performed under different conditions, since the results could be influenced by the vaccine strain and antigen payload used and also by the conditions under which the challenge studies were conducted (e.g. route of inoculation, virus dose, time of exposure – in case of contact challenge – and match of challenge) (Cox and Barnett, 2009). If the adjuvants are assessed for immunogenicity on target species, the ELISA for antibody quantification showed more reproducible results than virus neutralization (VN) tests (Van Maanen and Terpstra, 1989).1

Although many adjuvants have been developed and are available, the costs of testing novel adjuvants is prohibitive.

**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. However, it is important that potential new vaccine strains be tested for sensitivity to inactivation and well as antigen stability after inactivation.

**Vaccine Purification/Differentiating Infected from Vaccinated Animals (DIVA)**

In the 1990s, a major support to FMD monitor, control and eradication campaigns implemented in zones

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1 Adjusted from Chapter 12, Foot-and-mouth Disease Virus Current Research and Emerging Trends, Domingo & Sobrino 2016
or countries practicing vaccination has been the development and application of improved diagnostic tests that could detect FMDV infection regardless of vaccination (DIVA). The approach was based on the detection of antibodies to viral non-structural proteins (NSP) which presence is known to be associated with viral replication, and thus, in principle are induced only during infection and not after immunization with conventional high quality inactivated vaccines (De Diego et al., 1997). In contrast, detecting antibodies to viral structural proteins using conventional methods such as liquid or solid phase blocking ELISAs or virus neutralization are not an option, because these antibodies are produced in animals as a result of both infection and vaccination.

Due to the conserved nature of NSPs, infection with any serotype of FMDV can be detected with a single serological test. Consequently, the detection of antibody to the NSPs of FMDV has been very useful to identify past or present infection with any of the seven serotypes of the virus whether or not the animal has also been vaccinated. Therefore, these tests have been an important adjunct to substantiate freedom from infection on a population basis when identifying FMD-free zones and hence in supporting international recognition of free zones practicing vaccination (Bergmann et al., 1993; Paton et al., 2006).

When applying these tests during post-outbreak surveillance when vaccination was applied, in which case the main aim is to detect acute or persistent infection, especially subclinical infections, there are technical and statistical considerations. Acute infection cannot be detected by serology in individual animals until antibodies develop, so that paired serology would be needed. In addition, NSP tests can not differentiate between a persistently infected animal that transmits virus to others (carrier), from those that no longer present a risk of transmission, such as those animals that already recovered from the infection, or persistently infected animals that do not transmit virus. The latter could indicate eventual infection in the herd or in epidemiologically linked herds (Brocchi et al., 2006; Paton et al., 2006).

Added to the mentioned limitations, the identification of infection in individual animals would require sampling in principle almost all animals, if not all, using a perfect test, considering the low prevalence that can be expected (Brocchi et al., 2006; Paton et al., 2006). Various factors, many not test related, interact to affect the confidence with which disease freedom can be substantiated by serology. Guidance on how to carry out serosurveillance for FMD after outbreaks have been controlled is provided by the OIE Terrestrial animal health code and by the European Directive on FMD control.

In any case, a major prerequisite for the application of NSP testing is to ensure that the vaccines used in vaccination campaigns or emergency vaccination have been formulated using FMD antigens that have been purified to remove most of the NSPs, so that they do not induce antibodies to NSPs in the vaccinated 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. In addition, tests to determine the amount of NSP during the production process are also becoming available (Capozzo et al., 2010).

Multiple vaccinations may increase the probability of inducing NSP antibodies, but emergency use of vaccine in previously free countries will probably use only one or two doses of vaccine. Confirmation of vaccine purity may be shown by testing sera from animals vaccinated at least twice with the batch for
absence of antibodies to NSPs. Experience so far has indicated that high quality vaccines are sufficiently pure so that they do not interfere with the serological diagnosis of infected animals (Smitsaart et al., 2015).

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.

**Potency**

There are currently several versions of standards for potency testing FMD vaccines (European Pharmacopeia, 2009; Manual of Diagnostic Tests and Vaccines for Terrestrial Animals 2018; Chapter 2.1.8 (updated 2017). 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; De Clercq et al. 2008).

The potency of FMD vaccines according to the OIE Terrestrial Manual has traditionally been expressed as the number of 50 percent cattle protective doses (PD50) contained in the dose stated on the label. The PD50 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 ID50 (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 PD50 per dose of cattle. European Commission directives state that FMD vaccines must exceed the European Pharmacopoeia and should have an observed potency of 6 PD50 per cattle dose. The latest revisions of the OIE Code (OIE Code, 2017), also states that 6 PD50 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.

High variability was associated with the PD50 test, including overlapping confidence intervals and absence of statistical controls. 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 PD50 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 TCID50 (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
Potency tests in other target species, such as sheep, goats, pigs 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.

In addition to the lack of standardized methods to determine the potency of FMD in small ruminants and pigs, the challenge routes for all species need to be reevaluated. Currently the intradermological route is used for cattle, and although it provides accuracy regarding volume and infectious doses injected, it does not reflect the natural route of infection for FMD susceptible species. Methods such as aerosolized virus, intra-nasopharyngeal instillation have been shown to provide reproducible results and are easy to administer (Stenfeldt et al 2014; 2015a b). The dose of virus used should also reflect a natural challenge dose whilst ensuring repeatable results. This is an area that needs more research.

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 (Maradei et al., 2008; Periolo et al., 1993).

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 W/O emulsion vaccine remained seropositive with titers believed to be protective for at least 180 days after vaccination. A second dose applied 6 months after the first vaccination induced a significant rise of antibody titres which were maintained at high levels for more than 12 months after the second vaccine dose (Augé de Mello et al., 1980). Swine challenged with low levels of homologous virus up to seven months after a single vaccination displayed no clinical disease. In sheep and goats, high titres of neutralizing antibodies
were detected after a one-dose vaccination with single and double oil emulsion vaccines at 15–60 days which persisted for more than 5 months. Revaccination in pigs and sheep increased antibody titres that are maintained for 24 weeks or longer (Cox et al., 2003; Hunter, 1996; Liao et al., 2003; Patil et al., 2002; Selman et al., 2006; Späth et al., 2008). A booster regime is recommended to ensure high immunity under different field conditions. Therefore no tested vaccines have provided long term immunity and duration of immunity is an area that needs more research.

**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.

Several factors, such as age of the young animal, the level of immune response of the mother, and species determine the impact of maternally derived immunity on vaccination responses. In addition, there may be differences between adjuvants. In general, it is accepted that vaccination of young animals is advisable in endemic settings and despite the suppression of immunity due to maternally derived antibodies, vaccination may act as a prime. This is another area that needs more research.

**Vaccine Matching**

The new May 2017 version of the OIE Terrestrial Manual (OIE Code, 2018) is available on the OIE website (http://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/2.01.08_FMD.pdf) and includes a 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 IpELISA. 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 (Lavoria et al., 2012).

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; Horsington J et al 2015, 2018). However, these observations may not be true for all serotypes, and the biological bases for such results are still 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 animal diseases (i.e. equine influenza) strain in a short-term vaccine efficacy study. Daly et al 2007). In this scenario, however, the onset of full protection, if achieved, might be delayed compared with homologous protection.

Vaccine matching based on expected percentage of protection (EPP) values is widely used in South America where correlation tables are available for the vaccine strains used in the region. This test uses antiserum raised against a vaccine strain (primo and booster vaccination). The titres of sera against 100 TCID50/100 µl of sera/virus mixtures of the homologous vaccine strain and the same dose of a field isolate are compared to estimate the immunological coverage of the vaccine strain in relation to the field virus (OIE Terrestrial Manual, 2018). Although the degree of the titre that relates with protection is not the same for different strains, in general titres over 2.1 and 1.6 for LPBE and VNT assays, respectively, can be considered as protective for most vaccine strains, which in overall results in an EPP close to 75% which, when a group of 16 vaccinated animals are used, is an indication that the vaccines will protect against the field strain.

**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 for emergency use during outbreaks. 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). Following this, several other countries now have reserves (Australia, New Zealand, etc) and the OIE has also established banks for specific regions. There is now also an agreement between several banks to share doses if required.

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.

The main advantage of vaccine banks is the availability of finished vaccine for immediate use in emergency vaccination. However, their disadvantage 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. In this context, and as part of the contingency plan, veterinary services may choose to use already formulated polyvalent or monovalent vaccines as a primary barrier to prevent the spread of the disease. Contracts with companies that already have an on-going production and sales in countries that practice vaccination are highly desirable (Roth and Spickler, 2014).

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 and continues until the present with a chapter dedicated to the standards required for vaccine banks (Chapter 1.1.10).

PRAGMATIST: A DECISION SUPPORT TOOL FOR FOOT AND MOUTH DISEASE VACCINE BANK MANAGERS

One of the critical aspects of a strategic vaccine bank is to have the appropriate selection of vaccine antigens to address disease outbreaks by unanticipated viral strains. It is very difficult and expensive to acquire and maintain vaccine antigens against all possible strains that might be accidentally or deliberately introduced to the country. Therefore, vaccine banks have moved toward risk analysis to determine the composition of their stored antigens. Recently, the EUFMD and the World Reference Laboratory for FMD (FMD-WRL) at The Pirbright Institute, have developed a computer tool called “Pragmatist”. The Pragmatist tool is used for prioritization of antigen management using international surveillance to support risk-based decision-making for vaccine banks and FMD preparedness. This tool is now used by the WRL to recommend FMDV vaccines for inclusion in vaccine banks but in the future...
can be adapted for use in endemic regions. For more information visit:

The technical advantages and disadvantages of antigen banks are summarized in Appendices X and XI, respectively.

**Alternative routes of vaccination**

All current FMD vaccines are administered intramuscular (IM). Although this is not the normal route of infection it stimulates both the B and T cell arms of the immune system. Work is currently being performed to investigate the use of intradermal vaccination in cattle (Pandaya et al., 2012) and pigs (Eble et al., 2009) and determine how this affects the immune response to vaccination. Very little work has been done looking at oral or nasal application of vaccine to stimulate a mucosal response and this is an area that warrants more research. In addition, alternative routes of vaccination may prevent tissue damage, a concern in livestock production (mostly for pigs in Asia).

**Summary of Obstacles to Vaccinating Against FMD**

- Conventional inactivated FMD vaccines cannot be manufactured outside BSL3 containment facilities.
- There is concern in both FMD-free and FMD-endemic countries that live virus may escape from manufacturing facilities, as has occurred in Pirbright, UK, 2007.
- 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. Some vaccine strains included in high potency vaccines could compensate for antigenic divergences.
- Countries have regulatory restrictions as to the strains that are allowed for importation. South America and India have significant restrictions and do not have access to all commercial FMDV vaccine strains. Recently, the Commission for the Control of FMD (COSALFA) considered the authorization to allow work on foreign FMDV strains under certain biosecurity conditions and requirements. Wageningen Bioveterinary Research, Lelystad in the Netherlands is the exception and allows all strains. The Pirbright Institute 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 persistently infected animals.
- 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 an area that may need more study.
- There is a resistance to oil adjuvants in Africa due to local reactions and the perceived higher cost of the vaccine. The current alhydrogel adjuvanted vaccines provide only short-lived immunity and therefore constrains control efforts.
- 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}$. The differentiation between the different doses should be abolished and
focus placed on high quality vaccines at a sufficient antigen dose to induce fast and long lasting immunity. This will also assist with cross-reactivity.

- 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) (Capuzzo et al., 2010). This test is commercially available in a kit format.
- Since production methods and quality control tests are considered confidential by the large majority of manufacturers, it is imperative that owners of antigen banks ensure they receive all the necessary information that guarantees a quality product.
- 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 previously free countries or zones after disease eradication will extend international trade prohibitions.

Assessment of Commercial Vaccines (See Appendix II)

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, with an expected global market reaching USD 3.0 billions by 2025. (https://www.grandviewresearch.com/press-release/global-foot-mouth-disease-fmd-vaccine-market). A list of FMD vaccine manufacturers and the vaccines produced worldwide is provided in Appendix XII. 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 Foot-and-Mouth Disease Countermeasures Working Group (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 Desired Vaccine Profile on Page 88).

Emergency Use (high potency) FMD Vaccines
- Several vaccine banks are in place such as the North American Foot and Mouth Disease Vaccine Bank (NAFMDVB), Australian Vaccine Bank; European Union bank, Argentinian bank, etc.” A cooperation agreement has been signed between a number of the banks with sharing arrangements.
- 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.

- Banks have different arrangements with commercial entities on how and where bulk antigens are stored and where it will be formulated and finished.
- Banks have agreements in place with varying times between initiating deployment and finishing vaccine from frozen antigen concentrates.
- Vaccines are extensively tested for early protection.
- Because normal batch or serial tests to demonstrate purity, safety, and potency require several weeks to complete, several banks have procedures in place to allow release of the emergency vaccine before results are available. Tests such as antigen content and emulsion parameters could be done rapidly.

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 persistently infected animals after FMDV challenge. Although with high levels of herd immunity the prevalence of persistently infected animals is low and their role in the generation of new outbreaks has not been demonstrated.
- 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).
- Enhancement of the immune response induced by the inclusion of saponin in oil adjuvanted vaccines has been reported (Sadir et al., 1999).
- Local reactions are a concern in many countries and either preclude the use of oil adjuvants or result in farmer resistance to use them. However, local reactions could be multifactorial (degree of antigen purification, type and quality of adjuvants, dose volume, host factors, vaccination practices, route of inoculation) and is an area that may warrant more research.

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:
  - are not very effective in swine
  - have a shorter shelf life than oil emulsion vaccines
  - is more sensitive to freezing
  - are less potent per ug of antigen
  - produce a shorter duration of immunity
  - interfere more with colostrum immunity

Other Novel adjuvant tested in laboratory setting for Killed FMDV vaccines

- Novel Acuose (Montanide ESSAI-EMS-D12802 VGPR) was tested with killed vaccine and showed protection at 4 dpv in cattle (Quattrocchi et al 2014)
- Montanide ISA was also effective in cattle (Dar et al., 2013. Vaccine 31:3327-32)
- Ni2+-chelated nanolipoprotein complexed with monophosphoryl lipid A (MPLA:NiNLP) has been evaluated as an adjuvant of the inactivated FMD vaccine in mice (Rai et al., 2016)
• Imidazoquinoline compounds, imiquimod (R-837) and resiquimod (R-848), known as TLR7/TLR8 agonists have been recently used in combination with poly (I:C) and alum to enhance humoral and cellular immune responses of inactivated FMD vaccine in mice (Zhou et al., 2014).

• Flagellin, a potent activator of the NF-κB, that signals through TLR5, has demonstrated some adjuvant activity for inactivated FMD vaccine in a guinea pig model (Hajam et al., 2013)

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.

Vector delivered FMD vaccines: Human Adenovirus 5 (Ad5)-vectored Adjuvanted FMD Vaccine Platform
Viral vectors are used to in vivo produce FMDV structural proteins with the aim of synthesizing virus-like particles (VLPs) inside the vector-infected cells and potentially induce both humoral and cell-mediated immune responses. Numerous groups have focused on the use of different viral vectors like vaccinia virus [Sanz-Parra 1999], fowlpox virus [Ma et al 2008], attenuated pseudorabies virus (PRV) [Zhang 2011] or “single cycle” Semliki Forest virus (SFV) [Gullberg 2016] to induce protection against FMDV. However, these platforms have not been tested systematically in the natural host or only displayed limited efficacy in livestock species such as swine or cattle.

To date, the most successful strategy to induce protective responses in animals has been the use of a recombinant-replication-defective human adenovirus type 5 coding for the FMDV capsid (Ad5-FMD). These vaccines are based on a human adenovirus C, serotype 5 (Ad5) genome containing deletions in the E1, E3, and E4 regions that 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.

These vaccines have been shown to protect swine and cattle from clinical disease as early as 7 dpv and for up to 42 dpv when challenged by the standard intradermal inoculation routes [Mayr 1999, Moraes 2002, Pacheco 2005, de Avila Botton 2006]. 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. An Ad5-FMD serotype A vaccine was furthered developed through a collaboration between the U.S. Dept of Homeland Security, the USDA and industry partner GenVec
Inc/Merial Inc. Pivotal minimum immunizing dose studies have been satisfactorily completed and a vaccine release dose has been approved by USDA CVB, leading to licensure for use in the U.S. (Grubman et al 2012; Schutta et al 2016). However, the Ad5-FMD approach still requires a relatively high dose to achieve protective responses resulting in high cost of production, which is problematic for a veterinary vaccine especially in developing countries. Recently, several strategies have been successfully used to enhance the potency of Ad5-FMD: co-delivery of full-length 2B coding region to improve synthesis of FMDV capsid proteins [Pena et al 2008, Moraes et al. 2011], inclusion of mutations in the 3C protease to decrease toxicity and improve FMD capsid expression (Puckette et al., 2017), addition of an extra RGD motif inserted in the HI-loop of the adeno fiber to target dendritic cells [Medina 2015] and formulation with adjuvants such as synthetic double-stranded RNA stabilized with poly-L-lysine and carboxymethyl cellulose (polyICLC) [Diaz-San Segundo 2014] or ENBL® (Barrera 2018). Industrial processing of Ad5-FMD has been accomplished and a defined product profile established (Brake et al 2012). Remarkably, it has been recently shown that combination of Ad5-FMD vaccine with Ad5 that deliver interferons (IFNs) either in the same [Su et al., 2013] or in separated vectors induced full protection as early as 1 dpv and for up to 21 days in cattle and in swine [Diaz-San Segundo et al., 2016; Moraes et al., 2003]. However, recent studies in cattle have shown that Ad5-FMD efficacy may be limited when multivalent preparations are administered as one inoculation in cattle (Sreenivasa BP et al., 2017)

Animals vaccinated with Ad5-FMD can be differentiated from animals infected with FMDV by using the recently developed FMDV 3ABC ELISA companion test (Chung et al 2018).

Ad5-FMD vaccine candidates developed since 2010 include Ad5-FMD serotype O Asia, additional A, and SAT2 vaccines. 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 other emerging genotypes are also planned by DHS S&T over the next few years.

Summary Assessment of Ad5-FMD Vaccine
One Ad5-FMD vaccine platform has been fully-developed and licensed in the US by the Department of Homeland Security (Ad5-FMD subtype A24). This 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) lacking the non-structural protein coding regions that allows for easy differentiation of infected from vaccinated animals (DIVA), 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) precluding 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 4-7 days post-vaccination (Schutta et al 2016, Diaz-SanSegundo et al 2016). USDA-CVB granted a license on the Ad5-FMD subtype A24 vaccine derived from master cells bank and master virus bank in 2012 upon completion of adjuvant safety studies and extensive clinical studies (Field Safety Study, Schutta et al 2016). Additional constructs has been produced and evaluated for purity, potency, safety, and efficacy. However, U.S regulatory approval by the CVB and environmental release under NEPA has not been done. This process typically requires 3-5 years to complete for each replication-defective recombinant vaccine. Additionally the Ad5-FMD platform has several unresolved issues and gaps that require further research including interference during multi-valent vaccination and scaling up of production for multiple FMDV serotypes.
DIVA marker cDNA-derived Killed FMDV Vaccine Platform (FMD-LL3B3D)

USDA-ARS scientists have developed a safe Foot and Mouth Disease (FMD-LL3B3D) vaccine platform for production of a marker inactivated vaccine and associated companion diagnostic test for the Differentiation of Infected from Vaccinated Animals (DIVA). This vaccine platform comprises a cDNA-derived attenuated FMDV backbone, molecularly and antigenically marked by deletion of the Leader protein and built-in negative epitopes in the non-structural proteins 3B and 3Dpol as DIVA markers. Further modifications are the inclusion of unique restriction endonuclease sites for rapid replacement of capsid coding sequences of different serotypes. Attenuation of the double negative marker virus was achieved by deletion of the virus Lpro coding sequence (Leaderless – LL), which is known to be a virulent factor involved in FMDV pathogenesis (Almeida et al. 1998; Brown et al., 1996; Chinsangaram et al., 1998; Mason et al. 1997, Uddowla et al. 2012). Animals infected with live FMD-LL3B3D by the aerosol and intra-dermolingual routes (cattle) or by direct inoculation in the heel-bulb (swine) demonstrated that the prototype virus candidate is highly attenuated, showed no signs of clinical disease and failed to spread to contact animals in both susceptible livestock models. Vaccine production methods for FMDV LL3B3D are similar to those currently established for inactivated vaccine antigen production. A main advantage of FMDV LL3B3D production is the possibility of a simplified downstream processing without the need for removal of non-structural proteins, as the DIVA antigenic markers are intrinsically present in the vaccine backbone. Another advantage of the FMDV LL3B3D is the fact that specific serotype and subtype vaccines can be designed and rapidly produced even in the absence of the available vaccine strain. Chemically-inactivated, oil adjuvanted, vaccines, consisting of 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). 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 tests already available in the market (3ABC cELISA).

Summary Assessment of DIVA marker cDNA-derived Killed FMDV Platform (FMD-LL3B3D)

This platform is under development by USDA-ARS scientists at the Plum Island Animal Disease Center in partnership with Zoetis Inc. 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 decrease in 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. In 2018, a select agent exclusion on the FMD-LL3B3D vaccine was granted by USDA-APHIS-Select.
Agent Program. Furthermore, the US Secretary of Agriculture has authorized the movement of the modified, non-infectious Foot-and-Mouth Disease virus candidate from the Plum Island Animal Disease center to the U.S. mainland for the purpose of continued research and vaccine development. Based on the FMD-LL3B3D vaccine 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

**Novel FMD technologies that could be applicable to multiple platforms:**

**His-tagged FMDV inactivated vaccine platform**
This technology consists in introduction of modifications to the FMDV capsid that allow direct binding of immunomodulatory molecules such as adjuvants. This technology has been applied to a novel chemically-inactivated vaccine platform was generated using a cDNA-derived virus that displays His-Tag on the capsid surface capable of binding metal ions. Novel Ni$^{2+}$-chelated nanolipoprotein complexed with monophosphoryl lipid A (MPLA:NiNLP) incorporated into a chemically-inactivated His-tagged-FMDV was shown to be a highly effective vaccine (Rai et al 2016)

**Mosaic FMDV vaccine platform**
One of the biggest challenges in FMD vaccination is the narrow coverage of vaccines within serotype. Recently, ARS-USDA at Plum Island in collaboration with Los Alamos national Laboratory (LANL) designed and developed serotype A “mosaic” vaccines with modified capsids that were capable of inducing broad heterologous protection against widely divergent serotype A challenge viruses. This technology is the subject of a recent US patent (US patent application Serial N0 15/785,875 filed 10/17/2017).

**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 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 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 a dendrimerric 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. (Blanco et al 2016, Soria et al. 2017, 2018)

**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.

**Alternative Subunit and Virus-like Particle (VLP) Platforms**

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 structural proteins of the virus, but lack the viral genome. 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 experimental VLP vaccine for FMD 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 and FMDV VP1 (Shi, *et al.*, 2006), IL-18 (Shi, *et al.*, 2007) or HSP-70 (Su, *et al.*, 2007) has been shown to enhance SN and CMI responses in mice, however no livestock vaccine efficacy studies have been reported.

Baculovirus-derived experimental VLP vaccines for FMD can afford some protection against clinical disease in swine, but fail to limit viral replication (Grubman, *et al.*, 1993). Similar results using an *E. coli*-derived experimental VLP vaccine were also reported (Grubman, *et al.*, 1993). Studies using baculovirus-infected silkworms for the expression of P12A-3C coding sequences of an Asian FMDV
strain resulted in the production of VLPs which have 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. In more recent reports Porta and co-workers demonstrated the efficient production of VLPs by the baculovirus expression system by removing the toxicity of the 3C protease encoded by the P1-3C expression cassette (Porta et al., 2013a). Cattle immunized with wild-type of stabilized A22 capsids conferred partial protection against homologous challenge infection (Porta et al., 2013b). 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. The hepatitis B VLPs can be loaded with foreign peptides that can be presented on the surface of the capsid. 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 reported similarly to that of intact FMD particles (Clarke, et al., 1987). The formation of VLP in mammalian cells by modified HBc fused with specified FMDV multi-epitopes was also 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 experimental subunit vaccines for FMD 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). Recently, modifications of FMDV P1-2A mutants subunit expressed in plant were designed to increase capsid stability, hence they have shown to negatively impact their acid stability compared to the parental control (Veerapen et al 2018). To date, none of these transgenic plant-derived experimental subunit vaccine candidates have 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**

The VLP platform is still in the discovery phase and requires more time and effort to advance a candidate into targeted advanced development. The majority of the experimental VLP vaccines for FMD 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, insect cell culture systems are favored for relatively high yields and appropriate modifications and authentic assembly of VLPs. Before the practical utility of many VLP approaches for FMD vaccines, the yield should be improved to reduce the cost price for manufacturing, VLPs have to be prepared and evaluated for all relevant serotypes, and vaccine efficacy have to be enhanced to provide animals full protection.

**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 GFRA gap analysis working group 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 following section provides current needs 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 XII.

Current Needs for Diagnostic of FMDV

- Maximize the frequency and accessibility to in vivo observation of FMD infection in the natural hosts (APHIS and EuFMD trainings)
- 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. Lack of low-cost, easy to use medium to collect and preserve FMDV.
  - 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
- Platforms for high through put and rapid scaling up of diagnostics are needed to meet high influx of samples in face of an outbreak.
• A field validated serotype specific RT-qPCR is needed to rapidly identify the vaccination strategy
• Validated, well characterized biological reagents for diagnostics are needed:

Most urgent research priorities were defined as follows:

1. There is still a need to understand the mechanisms of virus neutralization, epitope mapping, and protein structural constraints governing immunological recognition in order to design proper diagnostic tests.
2. Determine what factors define protection and broad of coverage, like for instance to know what the best method is to reach “full avidity capacity” of the antibody response when using either high payloads or multivalent vaccines.
3. More information from current outbreaks and its relationship with the vaccines used in the region
4. Increase and share data from in vitro neutralization assays to reach levels of statistical analysis of results.
5. Coordinate vaccine sera production between laboratories so they are produced in large amounts, well characterized and directly related to the commercial vaccine seeds. This would not only harmonize and provide uniformity in the test but save resources.

Still pending gaps from 2010 gap analysis identified as a priority were:

1. Validation of diagnostics methods: methods must be tested in the field, isothermal tests need field validation data, and portable equipment need to be validated before being used in emergency situations; samples for performing validation tests are not accessible.
2. Diagnostic methods to identify preclinical, subclinical and unapparent infections (carriers) need to be developed and tested, as well as aggregate sampling methods (air filters, rope).
3. Research is needed for finding low-cost, easy to use, preserving medium to collect and transport FMDV. For example, there might be existing technologies that allow sampling and preservation such as dry swabs, or filter paper, or nanoparticles that could specifically concentrate and remove virus from inactivating agents.
4. Multiplexed assays are not yet fully developed and validated, to rapidly identify both infection and infecting strain in the field.
5. High-throughput and practical assays to measure levels of post-vaccination protective immunity against different virus strains, that can be easily adapted to any field strain
6. Improve serological methods for vaccine matching
7. Investigate the use of artificial intelligence for the development of algorithms to recognize FMD signatures in domestic animals
8. Detection (and accurate definition) of carrier status

Surveillance and early response
The first line of defense against an FMD outbreak relies on reporting all suspicious cases observed by personnel handling or interacting with susceptible animals such as farmers, technicians, farm hands, and veterinarians. In US, a certified Foreign Animal Disease Diagnostician (FADD) investigates suspicious cases and ships appropriate samples to the National Veterinary Services Laboratory (NVSL) Foreign Animal Disease Diagnostic Laboratory (FADDL), USDA-APHIS in Plum Island, and occasionally in duplicate to the local certified NAHLN for immediate screening and ruling out of FMD.
Below are listed tests for surveillance and tests for early and sustained response 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, antibodies against structural or non-structural proteins or nucleic acids.

**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-Lovett 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.

There are cheaper devices now in the market, making the technology affordable [http://www.flirthermography.com/cameras](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 detection 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.

Two commercially-available portable air sampling devices, the BioCapture 650 and the BioBadge 100, have successfully detected airborne virus in three proof-of-concept experiments involving pigs and cattle infected with FMDV (Ryan et al.; 2009).

**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, and Interdigital space. 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.

In cattle FMD is usually acute and relatively unmistakable. Vesicles, which may affect the mouth and/or feet and/or teats. Slavering and chewing movements associated with mouth lesions, foot tenderness, lameness and/or foot shaking, associated with foot lesions. They also develop fever, dullness and depressed appetite.

In sheep and goats clinical signs may be less obvious and clear-cut, however the occurrence of a sudden-onset severe lameness in several animals at one time is suspicious. Sudden death in apparently health lambs, abortions, and adult sheep which are listless and off their food may also be suspicious signs, particularly during an FMD outbreak.

Pigs develop severe lameness, high temperatures and frequently lie down. Lameness is more likely to be obvious if the pigs are on concrete or other hard surfaces than if they are on straw or similar soft bedding. Piglets may die suddenly without previous clinical signs. Vesicles may be variable in size, from small to quite large, and may develop on the coronets, mouth and snout and teats.

**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, always
considering that a positive result is valuable for quarantine and first response but needs to be confirmed in laboratory, and that a negative result lacks official reliability. In all suspect cases, there may be an additional interest to perform differential diagnosis from other diseases causing vesicular lesions in livestock, such as Seneca Valley A (SVA), Vesicular Stomatitis (VS), Vesicular Exanthema of Swine (VES), and Swine Vesicular Disease (SVD). The desirable general criteria for tests used in the field have been defined as “1C+4S+1P”, meaning Cheap, Speed, Specific, Sensitive, Simple and Pen-side/Digital.

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. Although many of the rapid assays initially used to detect FMDV do not identify the specific serotype causing the outbreak, the same sample or RNA can be used for further laboratory analysis and recovered from the lateral-flow stick. The pen side test is considered a valuable tool once the outbreak has been declared, otherwise only the reference lab can rule out as FMDV is an OIE reportable disease.

**Virus detection**

*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 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.

- Pen-side tests: Used to detect NSPs directly from Fluid from vesicles or from mucosal tissue, ruptured lesions a or saliva. The easiness of the test procedure and fast availability of an accurate result after 10 min shall enable fast and informed decision making. SVANODIP FMDVAG kit can also be used after treating with SVANODIP® FMDV-Ag Extraction kit to isolate Foot- FMDV from mucosal tissue when fluid from vesicles is no longer available. Together with the SVANODIP® FMDV-Ag pen-side-test FMD can be diagnosed even after acute onset of disease. A positive result is reliable but a negative result needs to be confirmed in the laboratory. They are useful for transporting the virus to the laboratory for further studies. They detect antigen of any strain. Validation in ongoing.

**Molecular assays**

*Available now*

- *Lab-based real time (rRT-PCR):* Real-time PCR is considered to be a practical tool in the presumptive diagnosis of FMD. The World Organization for Animal Health has approved use of the 5´ UTR (Reid et al., 2002) and 3Dpol, (Callahan et al., 2002) RT-PCR assays to supplement conventional diagnostic methods. 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; 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).

- **Field rRT-PCR:** 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 Mobile/portable equipment, there are opportunities to deploy mobile rRT-PCR assays inside a vehicle, or in local laboratories, for rapid diagnosis of suspect cases. (rapid diagnosis in non-centralized laboratory) - 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 already in place (Madi et al., 2012; Abd El Wahed et al, 2013; Hwang et al., 2016).

There are portable real-time PCR platforms such as the T-COR 8TM and PanNAT capable of being compatible for FAD detection under “field conditions”. These units tend to be lighter and less complicated than standard PCR platforms (Hole and Nfon, 2019), with limited numbers of wells, and a quick time from prep to answer (usually listed as around one hour) when used with quick point of care extraction kits. These kits may be best used under mobile laboratory conditions.

- **Isothermal amplification:** RPA and LAMP: Rapid, real-time reverse-transcription recombinase polymerase amplification assay (RT-RPA), employs primers and exo probes, and can be performed at 42 °C. The assay takes 20 min, and the detection limit at 95% probability is around 15 copies per reaction and 0.326 TCID50/mL based on plasmid copy number and tissue culture infectivity titer. Assay concordance for RT-LAMP and RT-RPA was 86–98% and 67–77%, respectively, when compared to rRT-PCR, with discordant samples consistently having high rRT-PCR cycle threshold values (no false-positives were detected for any assay). There are two commercial RPA kits:

1. TwistAmp® nfo (rRT-RPA-nfo) that uses RT Transcriptor (Roche, Mannheim, Germany). Primers and probes were as previously published (Abd El Wahed et al., 2013). Reactions are performed in duplicate at 39 °C for 40 min, with inversion at 4 min to mix.
2. TwistAmp® exo RT kit (rRT-RPA-exoRT) rRT-RPA was performed using the TwistAmp® exo RT kit (TwistDx Ltd., Cambridge, UK), with primers and probes as previously published (Abd El Wahed et al., 2013). Reactions are performed in duplicate at 42 °C for 20 min using a Genie® II, with inversion at 5 min to mix. Reverse transcription loop-mediated isothermal amplification (LAMP) takes 45 min at 64°C.
A group in China employed a set of four primers targeting FMDV 2B (Chen et al., 2011). The assay showed higher sensitivity than RT-PCR. No cross reactivity was observed from other RNA viruses including classical swine fever virus, swine vesicular disease, porcine reproductive and respiratory syndrome virus, Japanese encephalitis virus. Furthermore, the assay correctly detected 84 FMDV positive samples but not 65 FMDV negative specimens (Howson et al, 2017). There is a kit available that performed with high sensitivity Toshiba Medical Systems Corporation [TMSC] (rRT-LAMP-T-wet).

- **Nanopore sequencing technologies:** Oxford Nanopore Technologies has developed the nanopore DNA sequencer, the MinION. The MinION is a portable, real time, long-read, low cost device that has been designed to bring easy biological analyses such as disease/pathogen surveillance. The whole procedure can be conducted with a mobile suitcase laboratory, which is easy to use at the point of need in endemic countries. In a recently published paper the FMDV-RNA extraction was performed in field conditions, directly from vesicular material with the Dynabeads SILANE Viral Nucleic Acid kit (Invitrogen, Darmstadt,Germany), reverse-transcribed and sequenced using Nanopore technologies (Hansen et al., 2019). The procedure was completed in 5 h including RNA extraction, reverse transcription, second-strand synthesis, barcoding, sequencing and data analysis enabling rapid and reliable FMDV serotyping. It has not been validated yet.

**In development**
- **Multiplex RT-PCR assays for vesicular disease “rule-out”:** Multiplex bead-based assays for using the Luminex format allows the simultaneous detection of FMDV and other vesicular look-alike viruses (SVDV, VESV, VSV, BVDV, etc.) that cause vesicular disease in livestock. 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 adequate 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. Multiplex rRT-PCR using fluorescent probes can also be used for reducing false negatives produced by the OIE-recommended rRT-PCR assays detecting either the 5’UTR or the 3D, thus targeting a single conserved region of the FMDV genome. These assays are susceptible to false negative results when genetic variations exist within the genome targeted by those assays (King et al., 2006). Furthermore, false negative results occur with each of these assays because they are serotypically biased, because the 5’UTR assay has greater sensitivity in the detection of type A isolates, and because the 3Dpol, has greater sensitivity in the detection of SAT isolates. While it is presumed that comprehensive detection of FMDV can be achieved by using the 5’ UTR and 3Dpol, assays in combination, isolates (such as the IRQ 5/94 isolate) exist that can escape detection by both of these assays (King et al., 2006). Considering that failure to detect FMD can result in considerable spread of disease and major economic losses, it is important to consider other novel real-time diagnostic strategies for detection of FMDV, including those that probe for multiple genomic regions that would improve diagnostic sensitivity (Tam y col. 2009).

- **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). It has been used for FMDV genome detection by Baxi et al. 2006. This microarray-based test uses an FMD DNA chip containing 155 oligonucleotide probes, 35–45 base pair (bp) long, virus-common and serotype-specific, designed from the VP3-VP1-2A region of the genome. A set of two forward primers and one reverse primer were also designed to allow amplification of approximately 1100 bp of target sequences from this region. A total of 23 different FMDV strains representing all seven serotypes were detected and typed by this FMD DNA chip.

- **Serotype Pen-site qRT-PCR kit:** iiPCR Specific Reagent uses the insulated isothermal PCR (iiPCR) technology for detecting the specific nucleic acid sequences of target pathogens and specific genes. There is a kit on the market. It is example of hybrid real time system. This technology uses one constant temperature heating element to drive thermal migration of fluid in the reaction chamber to give a cycle of heating, cooling and reheating to give naturally occurring PCR cycles. The timeframe is about an hour to results. Some validation studies have been done but results were not comparable to regular PCR “POCKIT” that uses a portable equipment to run the assay in the field without RNA extraction needed. POCKIT results are available in 2 hours. It can be used for FMDV O, A and Asia 1. It has not been validated for other serotypes and it is not fully automated (Ambagala et al. 2016).

- **Superfast pen-site isothermal RT-RPA assay:** This is a visible and equipment-free reverse-transcription recombinase polymerase amplification assay combined with lateral flow strip (LFS RT-RPA) developed to detect the FMDV using primers and LF probe specific for the 3D gene. The results are obtained in 1 hour, by incubating the sample at 39C. Sensitivity is moderate (about 1000copy/reaction) and specificity (tested against PPRV, SPV, OrfV) is acceptable. The assay still requires RNA extraction. There is commercial kit in China and was used for detection of FMDV O, A and Asia 1, but it has only been validated only for type O. (Li et al., 2018).

**Serological assays**

*Available now*

- ELISA kits are commercially available to detect antibodies against Non-structural proteins “NSP”. Most of them are indirect species-specific tests (UBI, Boehringer Ingelheim Svanova, Panafotsa, Elabscience, CUSABIO, among others) or multi-species blocking ELISAs as the Priocheck (ThermoFisher), VMRD 3ABC ELISA, IDEXX and ID SCREEN® FMD NSP competition (ID-Vet). Comparative performance of UBI, PANAFTOSA and SVANOVA 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%.
• Good results have been achieved with detection of anti-NSP antibodies and type O strains (Chain et al, 2009) The procedure involves centrifuging the blood and placing three drops of sera onto the sample hole on the device and waiting 5-10 minutes to see if a “test” line develops on the strip. The strip must be read in this 5-10 minute window, as allowing development past the 10 minutes will give invalid results. A significant validation is still required. Lateral flow assays for antibodies have not yet validated for international standards.

• **FMDV NSP 3D antibody ELISA**: A liquid phase blocking 3D ELISA has shown good results in the 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. 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 seroconversion 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. Cedi also developed a version of this 3D ELISA that is not offered in ThermoFisher’s portfolio.

*In development*

• In South America, EITB assay, a Western blot test using recombinant non-structural proteins have been extensively used. It was produced by Panafosta. However, this assay was sometimes difficult to interpret (Bergmann et al. 2000). an improved EITB for confirmatory NSP that uses all NSP of FMD expressed with the recombinant baculovirus system, with altered molecular weights is being developed to make much easier the reading of results.

• **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 (Nfon et al, 2018). 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. The 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.

**Diagnostics in response to an FMD disease outbreak**

**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.

**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 complete genome, whole coding region (ORF), or just capsid proteins P1 or VP1 coding region of the FMD genome.
  
  - Sequencing using NANOPORE technology (see details above)

*In development*

- Serotyping by using multiplex PCR (see details above)

  - 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., 2017). 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.
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.

In development

- **Multiplex lateral flow strip test for FMDV based on monoclonal antibodies.** The multiplex-LFI strip test detects all 7 serotypes and specifically identifies serotype O, A and Asia 1 in field isolates using tissue suspensions and swabs. The sensitivity of this strip test is comparable to the double antibody sandwich ELISA for serotypes O and A, but lower than the ELISA for serotype Asia 1. The multiplex-LFI strip test identified all tissue suspensions from animals that were experimentally inoculated with serotypes O, A or Asia 1 (Morioka et al., 2015).

- qRT-PCR-VNT for rapid detection of neutralizing antibodies against FMDV, avoiding the need of visual detection of cytopathic effect. Results can be released in 24h. It has been validated only for type O (Lanzhou Veterinary Research Institute, 2018)

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. Then, vaccine matching is done for different purposes (i.e.): to QC a vaccine lot, to find the best available vaccine for a known outbreak, or just for preparedness (stockpile) to fight an unknown or emerging virus.

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 (2d VNT), ELISA (various serotype specific to measure total antibodies) and sequence analysis of VP1 or 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. Serological matching of field isolates to vaccine strains requires that isolates have been serotyped and grown in sufficient volume and titer using either primary or secondary cell cultures. The serotype is usually determined by Ag ELISA or CFT using reference type-specific serological reagents, although methods based on monoclonal antibodies or genetic typing may also be used.

Vaccine selection is currently informed by serological tests that measure the antigenic similarity between vaccine strains of FMD and field isolates of the virus. Vaccine matching tests measure how much an antiserum made against a vaccine strain will cross-react with another virus of the same serotype. Virus neutralization tests (VNT) and/or a liquid phase blocking ELISA (LPBE) are most commonly used, with the VNT considered the better correlate of protection but suffering from poor reproducibility.

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 (r1) with a vaccine antiserum, rather than two way testing (r2) which also requires an antiserum against the field isolate to be matched. The seroneutralizing assays are only possible in stablished cell lines (BHK-21 or IBRS-2) 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. 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).

Vaccine matching tests ignore the impact of vaccine potency, which together with match are key determinants of vaccine-induced protection. This means that a matching test addresses the question: "is this vaccine strain likely to be able to induce protection against a specific field virus if made into a moderately efficacious vaccine". This can be useful in a tender specification, but if a vaccine is already available, an answer to a different question is required, namely: "is this particular vaccine likely to protect against a particular field virus". This is better answered by a simpler measurement of how much antibody is induced by the vaccine against the heterologous virus, which takes into account both potency and match. This is useful for deciding if vaccine in a bank is likely to be efficacious and also to monitor protective levels of antibody in a vaccinated animal population.

For the last seven years, many efforts have been concentrated into the improvement of in vitro methods to measure protection in vivo. Since the last meeting in 2010, the evidence of lack of trust in the use of r
values to evaluate vaccine matching has become substantial and has even deemed unsuitable to evaluate vaccines of SAT serotype. Two alternative serological ELISA tests have been developed in Argentina (Capozzo et al., 1997; Lavoria et al., 2012) that measure antibody avidity and isotypes that appear to provide a better correlation with protection than VNT, whilst having a good reproducibility (Brito et al, 2014). Both require purified antigens to be prepared for each field virus against which protection is to be measured. These antigens are coated onto ELISA plates and then anti-vaccine antiserum is added from cattle previously immunized with the vaccine to be assessed. In the avidity test, the amount of bound antibody is compared with and without urea treatment, whilst in the isotype test, the amount of bound IgG1 and IgG2 is measured. Compared to VNT, another advantage is that the test can be carried out with inactivated virus antigens outside of high containment.

The papers by Lavoria et. al and Brito et al proposed to use not only one test but an algorithm integrating different tests. Combining current tests to carry out vaccine matching ranging from sequence analysis, in-vitro vaccine matching, to ELISA avidity tests with other selection/predictive tools such as the genetic database and epidemiological information available through OIE/FAO laboratories, will allow to easier to make decision easier about what strains are the highest risks to a given country in order to select a vaccine antigen.

One important fact is the final awareness that using high payload monovalent vaccines in our in vitro tests may not be reflecting real world situations. In most cases the vaccines used are multivalent, and the field variants are evolving under the immune response (selective pressure) of a wide spectrum of antigens, boosters, different adjuvants, etc. The vaccine matching tests are currently made by monovalent sera obtained in naïve animals with vaccinated high payload immunogens and never boosted. The A/Agentina 2001 (Matton y col., 2004) and O1/Ecuador 2010 (Duque et al, 2016) are good examples in support of this lack of consistency of in vitro vs. in vivo results.

**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 titers 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 neutralization test:** This test uses an antiserum raised against a vaccine strain. The titers 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.

- **Avidity ELISA**: The quality of vaccine-induced antibodies in terms of avidity has been identified as a determining factor in efficacy. Avidity is defined as “functional affinity” describing the interaction between an antibody and the bound antigen, and it is influenced by the amount of antibodies, their diversity and affinity. When vaccines stimulate the acquired immunity, antigen-specific B cells undergo somatic hypermutation and affinity-based selection, resulting in B cells that produce antibodies with increased avidity over germline antibodies. Thus, avidity can be considered a landmark of efficient vaccination. Avidity of antibodies can be easily measured by ELISA; when low-avidity antibodies are present in a sample, the OD values obtained are significantly reduced by treatment with urea. To provide an objective evaluation, the avidity index (AI) is calculated from the values with and without urea treatment.

The protocol developed for assessing anti-FMDV antibody avidity uses 96 well flat bottom well plates that are coated with 50ul per well of a dilution that contained a pre-established amount of sucrose-gradient purified inactivated FMDV 146S particles in carbonate/bicarbonate buffer pH 9.6. Serum sample are then added in two-fold serial dilutions starting at 1:20 and incubated at 37°C for 1 hour. The procedure can be further optimized to perform a single dilution of the sample, enabling testing up to 44 samples plus controls per ELISA plate. Serum samples are washed twice with PBS and subsequently washed with PBS–7 M urea for 15 min at room temperature and followed by two regular-PBS washing steps. FMDV-specific antibodies are detected with HRP-labeled anti-bovine conjugate. The colorimetric reaction is revealed using a peroxidase chromogen/substrate mixture (ABTS/H2O2 or TMB). OD readings are corrected by subtracting mean blank OD values (cOD). The avidity index (AI) is calculated as the percentage of residual activity of the sera relative to the OD of the untreated (not washed with urea) sample.
• **Isotype ELISA**: Isotype ELISAs are indirect tests to titrate anti FMDV IgG1 and IgG2 in sera. It has been set up for bovine, swine and buffalo serum samples. The protocol entails the use of purified virus bound to the plate, incubation with serial dilution of the sera and revealing with anti-IgG subtype specific conjugates. The IgG1/IgG2 ratio is helpful for those samples with low level of antibodies (Lavoria y col. 2012), on which avidity cannot be computed. Infected animals elicit IgG1 levels before IgG2 is induced and even for homologous protection, higher IgG1 than IgG2 levels are found in protected animals even though total or neutralizing antibody titers are low (Capozzo et al., 1997; Pega et al, 2013-2015).

• **Interferon-Gamma (IFN-γ) in stimulated plasma**: The assay system has proven to be a rapid, sensitive and inexpensive method for measuring antigen specific cell-mediated reactivity when compared with the more traditional lymphocyte proliferation assay. The production of IFN-γ by stimulated helper T lymphocytes regulates production of immunoglobulins in FMD vaccinated animals (Grant et al, 2016). These demonstrated that virus neutralizing antibody titers in cattle vaccinated with an inactivated FMD commercial formulation were significantly reduced and class switching delayed following in vivo CD4(+) T-cell depletion (Grant et al, 2016). It has also been suggested that cell-mediated immunity may be involved in the clearance viral infection so its importance may reside in part from its potential ability to inhibit viral replication directly, although further work is needed to support this hypothesis.

Other reports have also demonstrated that IFN-γ responses are highly cross-reactive between serotypes as well as dependent on capsid integrity (Bucafusco et al., 2015), thus making this assay inadequate to test antigenic matching between strains. Moreover, INF-γ is measured using stimulated plasma, and requires viable cells, then fresh blood must be used (no more than 18h from withdrawal) and cultured ay 37°C with 5% CO2, which may complicate testing logistics. Some authors have used this assay to sort out protection status of animals with low levels of total antibodies (Parida et al, 2006), anyway and although some improvements have been made, the role of IFN-γ responses is still unclear.

**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.

**Tests for early and sustained response**

These tests are the same used to detect infected animals and should be applied to 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).

**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.

**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).

**SP antibodies**
*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. 2010). 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.

There are commercial antibody kits against O, A and Asia1 serotypes. They are blocking ELISAs (Priocheck) not aimed to quantify antibody responses, but can be adapted for that purpose. Other companies only provide Type-O ELISA (ID VET) or ASIA 1 “VDPro® FMDV Type Asia1 AB ELISA” (Median Diagnostics) and Elabscience.

*In development*
- **Isotype and avidity ELISA:** These two assays were developed for cross-protective responses. However, it is known that IgG1 is elicited shortly after vaccination (7 dpv) right after IgM which can be detected at 4 dpv (Pega et al., 2013).

**NSP antibodies**
Kits for NSP detection can be also used. They have been described in the previous section.
Recovery

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.

Available now
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 titer 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 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 Laboratories.

In development
IgG1 subtype ELISA using purified virus can be used for this purpose, as this isotype is induced shortly after vaccination, before IgG2 or IgA. Validation is needed.

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.

Available now
Most of the tests mentioned above for detection of anti-NSP antibodies can be used for this stage. 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 with a 4 h 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 h 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. The 3D ELISA is suitable in areas 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 h working day through automation of the test.

The NSP Priocheck blocking ELISA measures antibodies against 3B, so, if this test is used only this protein needs to be removed from the vaccine antigen preparation. 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.

Tests to monitor herd immunity
Validated assays that have been correlated with vaccine protection are very important tools. 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).

In development

- IgG1 and Avidity ELISAs: Avidity is a measurement of efficacious vaccine-induced immunity. High avidity antibodies derived from a successful and long-lived T-helper cell immune response, thus, reaching high avidity antibody levels is an indirect assessment of efficacious vaccination. Avidity ELISA can be used in a single dilution format enabling high-throughput assessments. Isotype and avidity ELISAs used purified 140S particles, while all others use inactivated cell-culture antigen. Depending on the inactivation treatment, whole particles from some labile strains can be disassembled and turn to 12S particles. In fact, it has been demonstrated that a 24h incubation of O1 Campos strain at 37°C results in a loss of 80% of whole viral capsids, while one 20% are lost for an A-strain (Bucafusco et al. 2015). This means that at least some of the currently used ELISAs may be detecting non-relevant antibodies against non-exposed epitopes present in 12S particles inside the
capsid, non-exposed in 140S particles. This fact can be one of the reasons of over-estimating the protective capacity of antibodies, and the measurement of less strain-restricted antibodies.

**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.

**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 and rRT-PCR is recommended. A recent publication show that qRT-PCR analysis of oral swabs is a useful approach in order to achieve a time efficient and reliable initial diagnosis of acute FMD in cattle and pigs, whereas probang sampling is essential for the detection of cattle that are persistently infected "carriers" of FMDV (Stenfeldt et al., 2016).
High-throughput serological assays are required to confirm freedom from disease after an FMDV outbreak.

**New diagnostic platforms with potential FMD applications**

- **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.

**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/β 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/β 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, i.e., 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 downstream 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).
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 GFRA Gap Analysis working group 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.

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.
- Established and emergent FMDV strains
- Development and standardized of tools to enable utilization of NGS-derived subconsensus sequence data for enhanced tracing
- Continued investigation of the relevance of subclinically infected animals in the propagation of contagion, including carriers and acute (neoteric) subclinical infections.

Viral Pathogenesis

- Continued investigation of determinants of virulence for different serotypes and strains of FMDV in cattle, sheep, pigs, Asian buffalo, and African buffalo.
- Continued investigation of virus-host interactions at the primary sites of infection in ruminants and pigs with focus on factors defining tropism, generalization, and early host responses.
- Elucidate viral and host mechanisms of FMDV persistence in ruminants with goal of identifying mechanisms which may be subverted through vaccines, countermeasures, or post-exposure therapy
- Determine characteristics and mechanisms of FMDV within-host evolution over distinct phases of infection
- Gain understanding of species-specific and breed-specific continuum of permissiveness/tolerance/resistance to clinical and sub-clinical infection
• Improved understanding of onset and duration of infectiousness from clinically and sub-clinically infected animals
• Elucidate viral and/or host mechanistic determinants of highly successful emergent lineages (PanAsia, Ind2001a-e)

**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 needle-free vaccine 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 adjuvants to improve the efficacy and safety of current inactivated FMD vaccines. Current oil adjuvant formulations may have undesirable side-effects and alum-based adjuvants may not be as 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

Countries that are currently FMD-free are 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 GFRA Gap Analysis Working Group 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 GFRA 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.
FIGURE 1: VIRUS STRUCTURE

Foot-and-Mouth Disease Virus

Partial cleavage products

+ Sense genome works as mRNA
• CAP-independent translation via Internal Ribosomal Entry Site
• Cleavage of polyprotein into S and NS proteins

Protease Cleavage Sites
Lpro ◆ unknown ■
2A □ 3cpro ▲

Viral particles
**FIGURE 2: VACCINE MATCHING**

Source: C.G. Schermbrucker (unpublished results)

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)

FIGURE 3: INFRARED THERMOGRAPHY

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.
## TABLE 1: FMD DIAGNOSTIC TESTS

<table>
<thead>
<tr>
<th>Test</th>
<th>What does it detect?</th>
<th>Development status</th>
<th>Capability</th>
<th>Utilization</th>
</tr>
</thead>
<tbody>
<tr>
<td>FMD real time RT-PCR (rRT-PCR) Test</td>
<td>Virus RNA</td>
<td>In use</td>
<td>Rapid diagnostics</td>
<td>Primary case identification, control, and surveillance</td>
</tr>
<tr>
<td>FMD Antigen-Capture ELISA</td>
<td>Virus protein</td>
<td>In use</td>
<td>Routine diagnostics and confirmatory testing</td>
<td>Primary identification /confirmatory test</td>
</tr>
<tr>
<td>High throughput FMD rRT-PCR Test</td>
<td>Virus RNA</td>
<td>In use</td>
<td>Large volume sample processing on semi-automated robotic systems</td>
<td>Surge capability, surveillance during an outbreak, response and recovery</td>
</tr>
<tr>
<td>Multiplex FMD rRT-PCR Test</td>
<td>Virus RNA - rule-out vesicular look alike disease</td>
<td>Feasibility testing</td>
<td>Rapid serotype identification, rule out look alike agents</td>
<td>Routine surveillance</td>
</tr>
<tr>
<td>Pen-side qRT-PCR</td>
<td>Virus RNA</td>
<td>Commercially available</td>
<td>Rapid diagnostics</td>
<td>Primary case identification in the field</td>
</tr>
<tr>
<td>Virus isolation</td>
<td>Infectious virus</td>
<td>In use</td>
<td>Routine diagnostics</td>
<td>Confirmation</td>
</tr>
<tr>
<td>Liquid-phase blocking ELISA (LPBE)</td>
<td>Serotype-specific antibody</td>
<td>In use</td>
<td>Confirming previous or ongoing infection in non-vaccinated animals and for monitoring immunity conferred by vaccination</td>
<td>Confirmation</td>
</tr>
<tr>
<td>Solid-phase competition ELISA (SPCE)</td>
<td>Serotype-specific antibody</td>
<td>In use</td>
<td>Confirming previous or ongoing infection in non-vaccinated animals and for monitoring immunity conferred by vaccination</td>
<td>Confirmation</td>
</tr>
<tr>
<td>NSP ELISA Serological Assay</td>
<td>Antibodies to non-structural proteins</td>
<td>Commercially available</td>
<td>Distinguish infected from vaccinated animals</td>
<td>Control and recovery phase surveillance</td>
</tr>
<tr>
<td>3D ELISA Serological Assay</td>
<td>Antibodies to non-structural proteins</td>
<td>Feasibility testing</td>
<td>Distinguish infected from vaccinated animals</td>
<td>Control and recovery phase surveillance</td>
</tr>
<tr>
<td>Nanopore sequencing kit</td>
<td>Virus RNA</td>
<td>Commercially available</td>
<td>Rapid diagnostics</td>
<td>Primary case identification in the field</td>
</tr>
<tr>
<td>Penside antigen test</td>
<td>Detection of FMDV antigen in swab and tissue samples</td>
<td>Commercially available</td>
<td>Penside test for rapid assessment of FMDV antigen</td>
<td>Detection of viral circulation during an outbreak without vaccination. Useful for confirmation in the lab by recovering virus and RNA from the device</td>
</tr>
</tbody>
</table>
APPENDIX I: 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 spreadsheet 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 GFRA Gap Analysis Working Group, 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 working group. 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.

<table>
<thead>
<tr>
<th>Weight</th>
<th>Critical Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>Efficacy</td>
</tr>
<tr>
<td>2</td>
<td>Safety</td>
</tr>
<tr>
<td>8</td>
<td>Available Today</td>
</tr>
<tr>
<td>10</td>
<td>Speed of Scale up</td>
</tr>
<tr>
<td>2</td>
<td>Storage</td>
</tr>
<tr>
<td>6</td>
<td>Distribution</td>
</tr>
<tr>
<td>8</td>
<td>Mass Administration</td>
</tr>
<tr>
<td>4</td>
<td>All Ruminants</td>
</tr>
<tr>
<td>6</td>
<td>DIVA Compatible</td>
</tr>
<tr>
<td>8</td>
<td>Dx Available</td>
</tr>
<tr>
<td>4</td>
<td>Cost to Implement</td>
</tr>
</tbody>
</table>
**ASSUMPTIONS**

**Vaccine Profile**

1. Highly efficacious: one dose 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. > 1 year duration of immunity
5. One week or less onset of immunity
6. No maternal antibody interference
7. Two years shelf life
8. Safe vaccine: non-abortogenic; all species; pure vaccine
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 strains

**Vaccine Administration**

1. Vaccine can be effectively deployed in the field (no cold chain required)
2. Accelerated vaccine delivery (need rapid individual animal inoculation)
3. Can vaccinate 10 million cattle in the first 4 weeks of an outbreak
# APPENDIX II: COMMERCIALLY AVAILABLE VACCINES

## Assessment of Commercial Vaccines, NVS FMD CWG, June 14, 2018

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

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<tr>
<td>8</td>
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<tr>
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<tr>
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<tr>
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<tr>
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<td>6</td>
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<td>Short withdrawal</td>
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</tr>
<tr>
<td>2</td>
<td>Feasibility of registration</td>
<td>8</td>
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<td>8</td>
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Rank each criteria 2, 4, 6, 8, or 10 on each criterion — no more than two "10" rankings allowed.

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<tr>
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<td>8</td>
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APPENDIX III: EXPERIMENTAL VACCINES IN THE RESEARCH PIPELINE

Assessment of Experimental Vaccines, NVS FMD CWG, June 14, 2018

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<td>6</td>
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Rank each criteria 2, 4, 6, 8 or 10 on each criterion – no more than two *10" rankings allowed

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## APPENDIX IV: VACCINE MATCHING

### Tests for FMD Vaccine Matching - GFRA, June 2018

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.

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<th>Ag CARTOGRAPHY</th>
<th>RCT</th>
<th>SPCE</th>
<th>LPRE</th>
<th>VNT</th>
<th>Avidity ELISA</th>
<th>IgG1/IgG2 ELISA</th>
<th>IFN-γ</th>
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Rank each criteria 2, 4, 6, 8, or 10 on each criterion — no more than two "10" rankings allowed.

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**Value**

| 252 | 188 | 218 | 192 | 208 | 258 | 236 | 284 | 282 | 176 |

- Commercially available (could be stock piled)
- In use in reference laboratories
- "Pipeline" technologies under development
# APPENDIX V: DETECTION

## Diagnostics For FMD - GFRA, June 2018

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<th>Weight</th>
<th>Critical Criteria</th>
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<th>Ag ELISA</th>
<th>LFD</th>
<th>LAB RT-qPCR</th>
<th>Mobile RT-PCR</th>
<th>LAMP</th>
<th>MicroArray</th>
<th>Sequencing (Lab)</th>
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<th>Seq</th>
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Rank each Criteria 2, 4, 6, 8 or 10 on each criterion – no more than two “10” rankings allowed.

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<th>LFD</th>
<th>LAB RT-qPCR</th>
<th>Mobile RT-PCR</th>
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<td>54</td>
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</tr>
<tr>
<td>Deployable to NA-ILN</td>
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<td>Rapid Result</td>
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<td>80</td>
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<td>Viral characteristics</td>
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<td>16</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>0</td>
<td>40</td>
<td>40</td>
<td>40</td>
</tr>
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<td>Easy to perform</td>
<td>12</td>
<td>30</td>
<td>48</td>
<td>36</td>
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<td>48</td>
<td>48</td>
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<td>36</td>
<td>12</td>
</tr>
<tr>
<td>Cost to Implement</td>
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<td>17</td>
<td>12</td>
<td>8</td>
<td>16</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
</tbody>
</table>

| Value                   | 340          | 390      | 472 | 600         | 494           | 440  | 244        | 428             | 324       |

- 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 - GFRA June 2018

<table>
<thead>
<tr>
<th>Weight</th>
<th>Critical Criteria</th>
<th>EITB</th>
<th>NSP 3ABC ELISA</th>
<th>Lab RT-PCR</th>
<th>sLFD</th>
<th>IgA</th>
<th>3D ELISA</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>Validation to purpose</td>
<td>6</td>
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<td>4</td>
<td>2</td>
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<td>Specificity</td>
<td>8</td>
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<td>10</td>
<td>4</td>
<td>6</td>
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</tr>
<tr>
<td>8</td>
<td>Sensitivity</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>4</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>8</td>
<td>Throughput</td>
<td>4</td>
<td>10</td>
<td>8</td>
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<tr>
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<td>Deployable</td>
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<td>8</td>
<td>8</td>
<td>10</td>
<td>8</td>
<td>3</td>
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<tr>
<td>4</td>
<td>Cost to Implement</td>
<td>8</td>
<td>8</td>
<td>5</td>
<td>8</td>
<td>4</td>
<td>3</td>
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</table>

### Rank each Criteria 2, 4, 6, 8 or 10 on each criterion – no more than two "10" rankings allowed.

<table>
<thead>
<tr>
<th>Critical Criteria</th>
<th>EITB</th>
<th>NSP 3ABC ELISA</th>
<th>Lab RT-PCR</th>
<th>sLFD</th>
<th>IgA</th>
<th>3D ELISA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Validation to purpose</td>
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<td>80</td>
<td>80</td>
<td>40</td>
<td>20</td>
<td>80</td>
</tr>
<tr>
<td>Specificity</td>
<td>80</td>
<td>80</td>
<td>100</td>
<td>40</td>
<td>60</td>
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</tr>
<tr>
<td>Sensitivity</td>
<td>64</td>
<td>64</td>
<td>64</td>
<td>32</td>
<td>16</td>
<td>48</td>
</tr>
<tr>
<td>Throughput</td>
<td>32</td>
<td>80</td>
<td>64</td>
<td>40</td>
<td>48</td>
<td>80</td>
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<tr>
<td>Pan-species use</td>
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<td>48</td>
<td>60</td>
<td>36</td>
<td>12</td>
<td>48</td>
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<tr>
<td>Deployable</td>
<td>24</td>
<td>48</td>
<td>48</td>
<td>60</td>
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<td>48</td>
</tr>
<tr>
<td>Cost to Implement</td>
<td>32</td>
<td>32</td>
<td>24</td>
<td>32</td>
<td>16</td>
<td>32</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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</tr>
</tbody>
</table>

| Value                     | 364  | 432            | 440        | 288  | 220 | 390      |

- 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 - GFRA, June 2018

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.

<table>
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<th>Weight</th>
<th>Critical Criteria</th>
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<th>VNT</th>
<th>SPCE - O/A/ASIA</th>
<th>LPBE</th>
<th>NSP 3ABC*</th>
<th>Lab RT-PCR</th>
<th>NSP 3D ELISA</th>
<th>sLFD</th>
<th>IgA</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>Validation to purpose</td>
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<td>6</td>
<td>6</td>
<td>4</td>
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<td>6</td>
<td>4</td>
</tr>
<tr>
<td>10</td>
<td>Specificity</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>8</td>
<td>Sensitivity</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>8</td>
<td>Throughput</td>
<td>8</td>
<td>8</td>
<td>8</td>
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<tr>
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<td>Pan-species use</td>
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<td>8</td>
<td>10</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>6</td>
<td>Deployable</td>
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<td>4</td>
<td>6</td>
<td>6</td>
<td>8</td>
<td>8</td>
<td>6</td>
<td>4</td>
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</table>

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

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<th>VNT</th>
<th>SPCE - O/A/ASIA</th>
<th>LPBE</th>
<th>NSP 3ABC*</th>
<th>Lab RT-PCR</th>
<th>NSP 3D ELISA</th>
<th>sLFD</th>
<th>IgA</th>
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</thead>
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<td>100</td>
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<td>64</td>
<td>64</td>
<td>64</td>
<td>64</td>
<td>48</td>
</tr>
<tr>
<td>Throughput</td>
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<td>84</td>
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<td>84</td>
<td>84</td>
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<td>48</td>
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<td>48</td>
<td>48</td>
<td>60</td>
<td>48</td>
<td>48</td>
<td>36</td>
</tr>
<tr>
<td>Deployable</td>
<td>36</td>
<td>0</td>
<td>60</td>
<td>24</td>
<td>48</td>
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<td>32</td>
<td>24</td>
<td>16</td>
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<td>432</td>
<td>412</td>
<td>412</td>
<td>518</td>
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</tr>
</tbody>
</table>

- **Commercially available (could be stock piled)**
- **In use in reference laboratories**
- **"Pipeline" technologies under development**
APPENDIX VIII: HERD IMMUNITY

### Diagnostics For FMD Herd Immunity - GFRA June 2018

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.

<table>
<thead>
<tr>
<th>Weight</th>
<th>Critical Criteria</th>
<th>VNT</th>
<th>SPCE</th>
<th>LPBE</th>
<th>SDLPBE</th>
<th>Avidity ELISA</th>
<th>Isotype ELISA</th>
</tr>
</thead>
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<tr>
<td>10</td>
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<td>8</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>8</td>
<td>Throughput</td>
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<td>8</td>
<td>8</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>4</td>
<td>ability to detect multiple strains</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>6</td>
<td>Pan-species use</td>
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<td>8</td>
<td>8</td>
<td>8</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>6</td>
<td>Deployable</td>
<td>0</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>6</td>
<td>Cost to implement</td>
<td>2</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
</tr>
</tbody>
</table>

Rank each criteria 2, 4, 6, 8 or 10 on each criterion -- no more than two "10" rankings allowed.

<table>
<thead>
<tr>
<th>Critical Criteria</th>
<th>VNT</th>
<th>SPCE</th>
<th>LPBE</th>
<th>SDLPBE</th>
<th>Avidity ELISA</th>
<th>Isotype ELISA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Validation for purpose</td>
<td>20</td>
<td>40</td>
<td>60</td>
<td>60</td>
<td>60</td>
<td>60</td>
</tr>
<tr>
<td>correlation to homologous protection</td>
<td>60</td>
<td>40</td>
<td>80</td>
<td>80</td>
<td>40</td>
<td>60</td>
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<tr>
<td>Throughput</td>
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<td>64</td>
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<td>80</td>
<td>80</td>
<td>80</td>
</tr>
<tr>
<td>ability to detect multiple strains</td>
<td>32</td>
<td>32</td>
<td>32</td>
<td>32</td>
<td>32</td>
<td>32</td>
</tr>
<tr>
<td>Pan-species use</td>
<td>48</td>
<td>48</td>
<td>48</td>
<td>48</td>
<td>36</td>
<td>36</td>
</tr>
<tr>
<td>Deployable</td>
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<td>48</td>
<td>48</td>
<td>48</td>
<td>48</td>
<td>48</td>
</tr>
<tr>
<td>Cost to Implement</td>
<td>12</td>
<td>36</td>
<td>36</td>
<td>36</td>
<td>36</td>
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<td>384</td>
<td>332</td>
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</table>

- **Commercially available (could be stock piled)**
- **In use in reference laboratories**
- **"Pipeline" technologies under development**
## 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

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

Rank each Criteria 2, 4, 6, 8 or 10 on each criterion — no more than two “10” rankings allowed

<table>
<thead>
<tr>
<th>Critical Criteria</th>
<th>Adeno-INF Type 1</th>
<th>RNAi/PMO</th>
<th>Receptor block</th>
<th>Polymerase inh.</th>
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</thead>
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<tr>
<td>Efficacy</td>
<td>60</td>
<td>20</td>
<td>20</td>
<td>40</td>
</tr>
<tr>
<td>Safety</td>
<td>60</td>
<td>60</td>
<td>60</td>
<td>20</td>
</tr>
<tr>
<td>One dose</td>
<td>60</td>
<td>36</td>
<td>24</td>
<td>12</td>
</tr>
<tr>
<td>Speed of Scaleup</td>
<td>32</td>
<td>32</td>
<td>32</td>
<td>32</td>
</tr>
<tr>
<td>Stability/Shelf Life</td>
<td>64</td>
<td>64</td>
<td>64</td>
<td>64</td>
</tr>
<tr>
<td>Storage/Distribution/Supply</td>
<td>48</td>
<td>48</td>
<td>48</td>
<td>48</td>
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<tr>
<td>Mass Administration</td>
<td>32</td>
<td>32</td>
<td>32</td>
<td>64</td>
</tr>
<tr>
<td>Ruminants/Pigs</td>
<td>12</td>
<td>12</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>Withdraw</td>
<td>48</td>
<td>36</td>
<td>36</td>
<td>12</td>
</tr>
<tr>
<td>Cost to Implement</td>
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<td>12</td>
<td>24</td>
<td>12</td>
</tr>
<tr>
<td>Value</td>
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<td>352</td>
<td>352</td>
<td>316</td>
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</tbody>
</table>
APPENDIX X: TECHNICAL ADVANTAGES OF ANTIGEN BANKS


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 to 10 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


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.
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REFERENCES


• Brown F. and Crick J. (1959). Application of agar gel diffusion analysis to a study of the antigenic structure of inactivated vaccines prepared from the virus of foot and mouth disease. J. of Immunology, 82, 444-447.


• Knight-Jones, T. J. and J. Rushton (2013). "The economic impacts of foot and mouth disease - what are they, how big are they and where do they occur?" Prev Vet Med 112(3-4): 161-173.

• Knight-Jones, T. J., M. McLaws and J. Rushton (2016). "Foot-and-Mouth Disease Impact on Smallholders - What Do We Know, What Don't We Know and How Can We Find Out More?" Transbound Emerg Dis.


• McGuire, T.C., Musoke, A.J., Kurtti, T., 1979, Functional properties of bovine IgG1 and IgG2: interaction with complement, macrophages, neutrophils and skin. Immunology 38, 249-256.


• Neilan, J., DHS S&T, unpublished observations. 2006.


• Pay, T. W., Hingley, P.J. 1987. Correlation of 140S antigen dose with serum neutralizing antibody response and the level of protection induced in cattle by foot-and-mouth disease vaccines. Vaccine 5, 60-64


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protein in lymphoid tissues of convalescent pigs does not indicate existence of a carrier state."
Transbound Emerg Dis 63(2): 152-164.

"Clearance of a persistent picornavirus infection is associated with enhanced pro-apoptotic and

• Stenfeldt, C., J. Arzt, J. M. Pacheco, D. P. Gladue, G. R. Smoliga, E. B. Silva, L. L. Rodriguez
and M. V. Borca, 2018: A partial deletion within foot-and-mouth disease virus non-structural
protein 3A causes clinical attenuation in cattle but does not prevent subclinical infection.
Virology, 516, 115-126.

antibody induced by immunization with a synthetic peptide is associated with protection of cattle
against foot-and-mouth disease. Immunology, 72, 99-103.

expression of FMDV immunodominant epitopes and HSP70 in P. pastoris and the subsequent
immune response in mice. Veterinary Microbiology 124, 256-263.

Adenovirus-Vectored FMDV Subunit Vaccine through Improving the Generation of T Follicular

• Summerfield, A., 2012: Viewpoint: factors involved in type I interferon responses during porcine

• Sun, P., S. Zhang, X. Qin, X. Chang, X. Cui, H. Li, S. Zhang, H. Gao, P. Wang, Z. Zhang, J. Luo
and Z., Li, 2018: Foot-and-mouth disease virus capsid protein VP2 activates the cellular EIF2S1-


• Sutmoller, P. and O. R. Casas (2002). "Unapparent foot and mouth disease infection (sub-clinical

• Sutmoller, P., Barteling, S.S., Olascoaga, R.C., Sumption, K.J., 2003, Control and eradication of

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