DRAFT

FMD Vaccine Surge Capacity for Emergency Use in the United States

A White Paper Prepared by the Center for Food Security and Public Health at Iowa State University for:

National Pork Board

National Cattlemen's Beef Association

National Milk Producers Federation

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EXECUTIVE SUMMARY

Foot and mouth disease (FMD) presents the greatest economic threat to U.S. animal agriculture and is viewed as the most important transboundary animal disease in the world. An outbreak of FMD in the U.S. would have a devastating impact on the U.S. economy extending far beyond animal agriculture. The structure of modern animal agriculture in the U.S., including extremely large herds and extensive intra- and inter-state movement of animals and animal products will make it nearly impossible to control an FMD outbreak in livestock dense areas without the rapid use of tens of millions of doses of FMD vaccine. The amount of antigen in the North American FMD Vaccine Bank is far below what would be needed to provide vaccine for a single livestock dense state. It would take many months to produce/obtain the volume of vaccine needed. Without sufficient vaccine to aid in the response, FMD could rapidly spread across the U.S., resulting in the destruction and disposal of potentially millions of animals, and become an endemic disease in livestock with spread potentially facilitated by deer, feral swine or other free-living animals. It would then require a much more extensive control program and could take many years to eradicate. Agriculture is critical infrastructure in the U.S. and cash receipts for livestock and poultry often exceed $100 billion per year. **Therefore, it is urgent to develop a plan to ensure that adequate supplies of FMD vaccine with multiple strains of FMD virus are rapidly available in the event of an accidental or intentional introduction of FMD virus into the U.S. This white paper is part of an effort by the private sector stakeholder community to work with the Secretaries of Agriculture and Homeland Security as directed in Homeland Security Presidential Directive 9 to develop a National Veterinary Stockpile (NVS) with sufficient quantities of FMD vaccine to protect U.S. agriculture, food systems, and the economy.**

The stakeholder community should form a working group to develop recommendations to be presented to the U.S. government for meeting the surge capacity needs for FMD vaccine mandated in HSPD 9. Potential solutions for meeting the surge capacity needs for FMD vaccine are summarized here.

Summary of Potential Solutions to Provide Adequate FMD Vaccine to Control a Type 3 or Larger Outbreak of FMD in the U.S.:

1) A combination of approaches can be used to assure surge capacity for FMD vaccines.

    a. Immediate Availability: Finished vaccine held in vendor-managed-inventory and ready for shipment within 24 hours.

        i. Enter into vendor-managed-inventory contracts with international manufacturers of FMD vaccines, for rapid delivery of multiple strains of finished vaccines into the U.S. All FMD vaccines that are licensed or permitted by USDA CVB for use in the U.S. and all FMD vaccines produced in the original E.U. member states (Maastricht Treaty; member states prior to 1994) that have either previously obtained EMA CVMP marketing authorization at the national level in one or more original E.U. member states, or single marketing authorization using the multi-strain dossier approach for use across all E.U. Member States could be considered to be safe and effective and pre-approved

for emergency use in the U.S. Contracts should be developed to provide enough vaccine to supply the U.S. until vaccine antigen concentrate (VAC) from the NVS is formulated into vaccine and available.

    b.   Short-Term Availability: Vaccine antigen concentrate (VAC) held in vendor-managed-inventory ready to be formulated into finished vaccine and shipped to the U.S.

        i.   Stockpile multiple strains of vaccine antigen concentrate (VAC) in the National Veterinary Stockpile (NVS). Enough VAC should be available for the period between depletion of the finished vaccine and availability of large amounts of vaccine available from production initiated at the beginning of the outbreak. The VAC should be held and managed by the manufacturer and the contract should support a rotating inventory (formulating the VAC into finished vaccine for sale and replacing it on a regular basis).

    c.   Long-Term Availability: Vaccine production initiated at the beginning of the outbreak for the specific outbreak strain(s) of FMD virus.

        i.   Enter into contracts with international manufacturers of FMD vaccines for surge capacity production of commercially available USDA licensed/permitted or approved E.U. licensed FMD vaccines.

        ii. Seek USDA licensure of new technology FMD vaccines that could be safely manufactured in the U.S. and which are based on a platform that allows various capsid serotypes/topotypes to be inserted into the vaccine. These would then be candidates for vendor managed inventory of finished vaccine and of VAC. Ensure that U.S. manufacturers have the surge capacity to rapidly produce finished vaccine at the beginning of an outbreak.

2) Ensure that all FMD vaccines used are capable of detecting infections in vaccinated animals (DIVA compatible), unless animals are intended for slaughter. Ensure that sufficient reagents and/or finished kits for DIVA testing will be available for the recovery phase of the FMD outbreak and sufficient NAHLN labs have been equipped, trained and proficiency tested to conduct this assay.

3) Develop and adopt available technologies and scalable information technologies for identifying and tracking all vaccinated animals and diagnostic testing results.

4) Develop interferon or other antiviral biotherapeutic products for inducing rapid and medium term resistance (1 day to 14 days) to FMD infection (a long term goal).

5) Form a standing advisory committee with expertise in FMD vaccines, production agriculture, economics, and emergency response to make recommendations on optimal use of vaccine as the outbreak unfolds.

6) Secure funds to enable the surge capacity need for FMD vaccines mandated in HSPD 9 to be met (estimated at $150 million/year for 5 years to help protect a $100 billion dollars a year (cash receipts) animal industry.

   As part of this effort, DHS S&T should conduct a classified Biological Threat Risk Assessment (BTRA) in collaboration with the USDA (APHIS and ARS), the Department of Commerce, and the Office of National Intelligence. The BTRA should include the size and economic scope of the livestock industry at risk; the potential sources of virulent FMD virus; the potential routes of incursion into the U.S. (both from natural and intentional introduction); the potential Foreign Terrorist Organizations (FTOs) with capability and interest to utilize FMD virus; an assessment of the ease of obtaining, transporting, and delivering virulent FMD virus; and the impact to the U.S. economy of an FMD outbreak in the U.S. (whether it be natural or intentional).

7) Convene a stakeholder community working group of experts capable of evaluating existing and new technology FMD vaccines under development to determine the technologies which can best meet the needs for emergency response vaccination in the US. The working group could enter into confidentiality agreements with biologics companies in order to have access to confidential business information which can inform the recommendations for incorporating existing and new vaccines into the surge capacity plan.

8) Conduct research into alternative delivery methods for vaccines which have been shown in cattle and swine to significantly reduce the antigenic mass required in each dose of vaccine, thus enabling existing or future VAC to be formulated into significantly more doses of vaccine.

1. STATEMENT OF THE PROBLEM AND APPROACH

Foot and mouth disease (FMD) presents the greatest economic threat to U.S. animal agriculture and is viewed as the most important transboundary animal disease in the world. An outbreak of FMD in the U.S. would have a devastating impact on the U.S. economy extending far beyond animal agriculture. Work on the Secure Milk Supply and Secure Pork Supply Projects by federal and state officials, industry and academia has made it clear that an FMD outbreak in livestock dense areas cannot be effectively controlled without the rapid use of tens of millions of doses of FMD vaccine. At this time, those doses are not readily available for U.S. use and it would take many months to produce/obtain that volume of vaccine. Without sufficient vaccine to aid in the response, FMD could rapidly spread across the U.S. infecting domestic and wild ungulates. An extensive control program would be required and it could take many years to eradicate the disease. **Therefore, it is urgent to develop a plan to ensure that adequate supplies of multiple strains of FMD vaccine are rapidly available in the event of an accidental or intentional introduction of FMD virus into the U.S. This is mandated in Homeland Security Presidential Directive 9.**

Homeland Security Presidential Directive 9 (HSPD-9, January 30, 2004) provides direction for "Defense of United States Agriculture and Food" (https://www.fas.org/irp/offdocs/nspd/hspd-9.html). "This directive establishes a national policy to defend the agriculture and food system against terrorist attacks, major disasters, and other emergencies." HSPD 9 directs that "the Secretary of Agriculture, in coordination with the Secretary of Homeland Security, and in consultation with the Secretary of Health and Human Services and the Administrator of the Environmental Protection Agency, shall work with State and local governments and the private sector to develop:

   a.   A National Veterinary Stockpile (NVS) containing sufficient amounts of animal vaccine, antiviral, or therapeutic products to appropriately respond to the most damaging animal diseases affecting human health and the economy and that will be capable of deployment within 24 hours of an outbreak."

The USDA has acknowledged that the amount of vaccine available in the North American FMD Vaccine Bank (which is controlled and shared by the U.S., Canada, and Mexico) is far below what would be required for an outbreak in a single livestock dense state. Since the need for vaccine in the U.S. is likely to be much greater than for Canada or Mexico, additional sources of FMD vaccines independent of the North American FMD Vaccine Bank are needed to adequately protect U.S. agriculture.

The funding USDA has and is receiving for the NVS is insufficient to provide adequate FMD vaccine stockpiles. An outbreak of FMD which occurred in a high livestock dense area such as Iowa, and which was not contained rapidly with stamping out, could quickly outstrip even the world's supply of emergency FMD vaccine. An FMD outbreak in South Korea depleted the banks of FMD vaccines from around the world in order to vaccinate a population roughly half the size of the livestock population in

Iowa. For an outbreak in Iowa, with over 20 million hogs and approximately 4 million cattle, the number of vaccine doses required (with two doses per animal) could easily exceed 50 million in a very short time. Insufficient vaccination capacity limits the ability of the US to be able to effectively respond with a vaccination strategy should that be the response choice made by USDA.[1]

There is clear desire on the part of many emergency management personnel to be able to use vaccination as part of the response to FMD. In a study in which potential incident commanders were interviewed about a Midwest FMD outbreak scenario lasting five weeks, 2 of 7 favored vaccination the first week of the outbreak scenario, and 6 of 7 wanted vaccination at some time during the 5 week scenario. One did not want to vaccinate ever during the scenario.[2] Rapid availability of large amounts of vaccine is very important for controlling an FMD outbreak. FMD models which estimate the size and duration of outbreaks are very complex, and provide a range of potential outcomes.[3] In a study using a model to estimate vaccination needs for an FMD outbreak in Minnesota, large scale vaccination (1,500 herds per day) reduced the size and duration of the outbreak if initiated within 21 days of the start of the outbreak.[4]

The need for additional supplies of FMD vaccine, as well as new vaccine approaches and technologies, to help meet this need has been recognized by USDA and DHS officials. USDA APHIS has had a series of meetings with stakeholders related to the need for and use of FMD vaccines and has funded the development of Secure Food Supply Plans that incorporate the use of FMD vaccine as an important control tool. USDA ARS and DHS S&T ChemBio Division have invested in research and development of new generation FMD vaccines. DHS S&T ChemBio division has also provided funding for FMD vaccines produced in other countries to be evaluated by USDA Center for Veterinary Biologics, for importation and sale in the U.S. in the event of an outbreak. In addition, DHS S&T funded a meeting on Vaccines and Diagnostics for Transboundary Animal Diseases, which brought together experts to discuss state-of-the-art technologies for improved vaccines and diagnostics for FMD and other high priority transboundary animal diseases.

This white paper is part of an effort by the private sector stakeholder community (National Pork Board, National Cattlemen's Beef Association, and National Milk Producers Federation) to work with the Secretaries of Agriculture and Homeland Security as directed in HSPD 9 to develop a National

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[1] Miller GY. Current vaccinology considerations in North American foreign animal disease events - Implications for foot-and-mouth disease (FMD) preparedness and response. In: Proceedings of the 117th annual meeting of the United States Animal Health Association. San Diego, CA. Oct 17-23, 2013.

[2] Parent KB, Miller GY, Hullinger P. Triggers for foot and mouth disease vaccination in the United States. Rev Sci Tech 2011; 30(30):789-96.

[3] Gale SB, Miller GY, Eshelman CE, Wells SJ. Epidemic simulation of a Foot-and-mouth disease outbreak in Minnesota. Submitted for publication.

[4] Miller GY, Gale SB, Eshelman CE, Well SJ. Emergency vaccination use in a modeled foot-and-mouth disease outbreak in Minnesota. Submitted for publication.

Veterinary Stockpile (NVS) with sufficient quantities of FMD vaccine to protect U.S. agriculture, food systems, and the economy.

The **Objectives of this white paper are to:**

1. Estimate the number of doses needed to control an FMD outbreak in the U.S. and the timeline when the doses may be needed as the outbreak unfolds.

2. Recommend the strains of FMD virus to be included in the NVS.

3. Review vaccine characteristics and vaccine production approaches that could meet the need

4. Review FMD vaccine and antiviral technologies currently available and under development, including:

   a. Commercially available killed vaccines produced in other countries that could be available nearly immediately.

   b. Vaccine banks containing vaccine antigen concentrate (VAC) which can be formulated into finished vaccine in a few days to weeks.

   c. New technology vaccines under development for production in the U.S.

      i. HAd5 vectored FMD vaccines
      ii. Leaderless killed FMD-LL3B3D vaccines
      iii. Alphavirus vectored FMD vaccines
      iv. Plasmid DNA FMD vaccines
      v. Baculovirus produced FMD vaccines (information to be added later)

   d. Interferon and other antiviral technologies

5. Propose approaches to ensuring sufficient vaccine availability for a Type 3 or greater FMD outbreak in the U.S.

6. Provide information that could be used to seek consensus among stakeholders, federal officials, and state officials on best mechanisms to ensure vaccine availability to minimize the economic, environmental, animal welfare, and food security impacts of a large FMD outbreak in the U.S.

2. CHALLENGES FOR CONTROL OF FMD IN THE US

The size, structure, efficiency, and extensive movement inherent in the United States livestock industry will present unprecedented challenges in the event of an FMD outbreak. No country with a livestock industry comparable to that of the U.S. has had to deal with an outbreak of FMD.

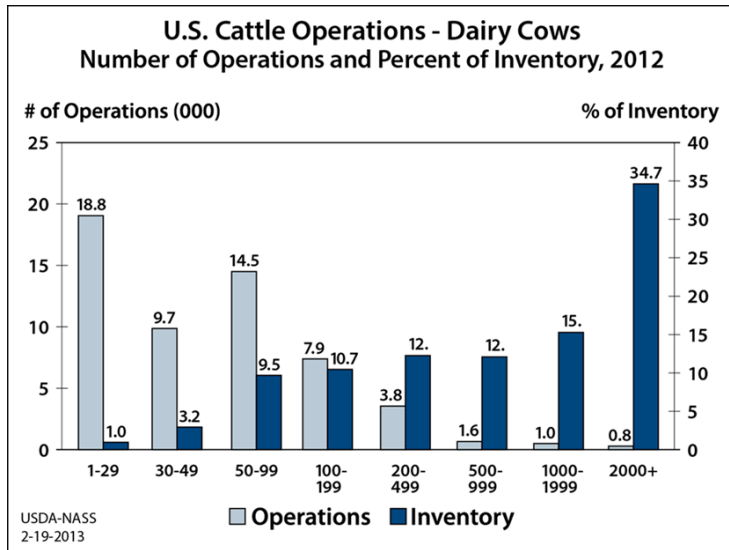| US Domestic Animal Population 2011			
Species	Population	Units	Establishments
Cattle	90,768,500	Animals	922,000
Sheep	5,345,000	Animals	80,000
Goats	2,862,000	Animals	151,000
Camelidae	122,680	Animals	26,060
Buffaloes	198,234	Animals	4,499
Swine	65,931,000	Animals	69,100
Cervidae	337,788	Animals	7,571
(from OIE World Animal Health 2011)			

The U.S. has some very large herds including feedlots with greater than 50,000 head of cattle, dairies with greater than 5,000 lactating cows, dairy calf ranches with greater than 70,000 head of calves (between 1 day and 4 months of age), and swine farms with greater than 20,000 sows. These premises are too large to rapidly depopulate to stamp out the disease. If it were possible to depopulate them, carcass disposal would present enormous environmental problems.
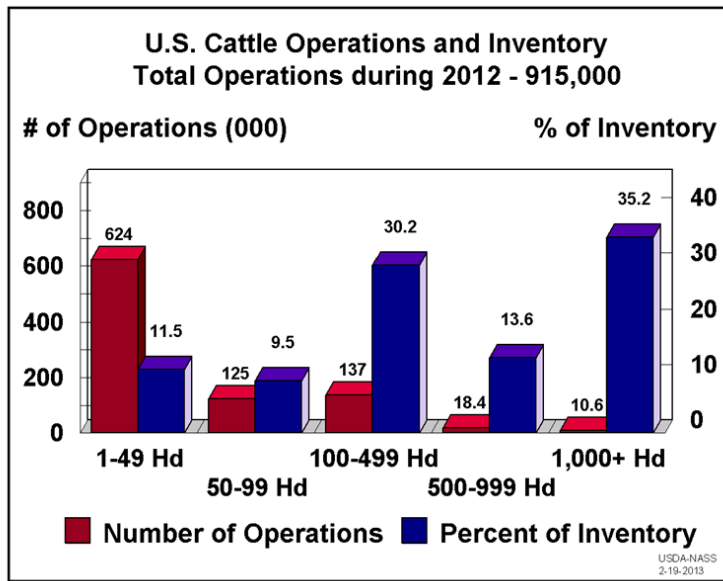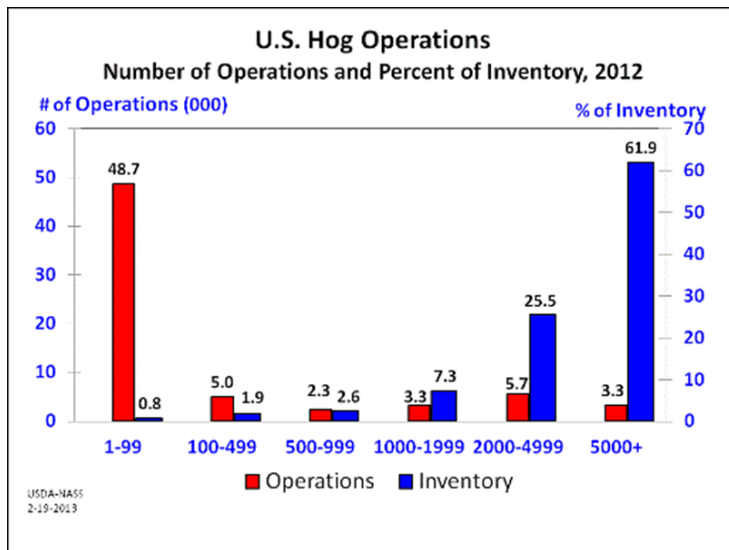
Livestock production in the U.S. depends on extensive movement of animals. It is estimated that approximately one million swine are on the road in trucks each day and about half of those animals are being sent to packing plants. Approximately 40 million swine are shipped into a new state each year (~110,000 each day). Many of those cross multiple state lines. This includes approximately 6 million swine from Canada shipped into the U.S. each year, and approximately, one million head of cattle per year that enter the U.S. from Mexico. Another factor is the extensive movement of people, feed, manure, and equipment on livestock premises each day. If FMD infection is not detected quickly, it is likely to spread rapidly due to extensive animal and related movements.
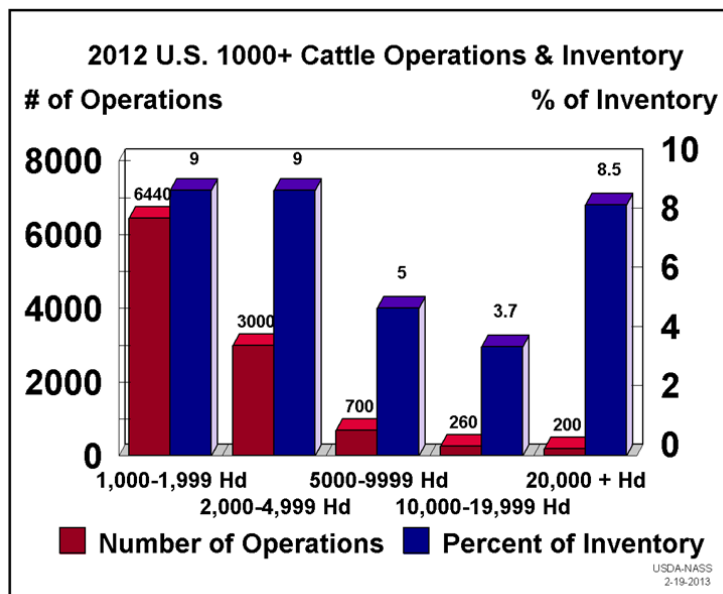
The diversity of herd size also presents problems in FMD control. In the U.S., 49% of hog operations have fewer than 100 head, whereas 62% of the inventory of swine is on operations with more than 5,000 head. Similarly, 18,800 dairy farms have less than 30 cows;

U.S. Cattle Operations - Dairy Cows
Number of Operations and Percent of Inventory, 2012

# of Operations (000)   % of Inventory

Operations: 1-29: 18.8; 30-49: 9.7; 50-99: 14.5; 100-199: 7.9; 200-499: 3.8; 500-999: 1.6; 1000-1999: 1.0; 2000+: 0.8

Inventory: 1-29: 1.0; 30-49: 3.2; 50-99: 9.5; 100-199: 10.7; 200-499: 12.; 500-999: 12.; 1000-1999: 15.; 2000+: 34.7

USDA-NASS 2-19-2013

■ Operations ■ Inventory

U.S. Hog Operations
Number of Operations and Percent of Inventory, 2012

# of Operations (000)   % of Inventory

Operations: 1-99: 48.7; 100-499: 5.0; 500-999: 2.3; 1000-1999: 3.3; 2000-4999: 5.7; 5000+: 3.3

Inventory: 1-99: 0.8; 100-499: 1.9; 500-999: 2.6; 1000-1999: 7.3; 2000-4999: 25.5; 5000+: 61.9

USDA-NASS 2-19-2013

■ Operations ■ Inventory

however, farms with more than 2000 animals account for nearly 35% of the U.S. dairy cow population. In addition there are an unknown number of "backyard" operations which maintain a few FMD susceptible animals. An FMD control program will need to include operations of all sizes. Small and large operations may face different challenges in FMD control. The small operations may not have good biosecurity, which may allow their animals to have contact with feral swine and deer. There are estimated to be more than 5 million feral swine and 30 million deer in the U.S. Large swine operations often have very good biosecurity, but depend on extensive animal movement on a regular basis. If animal movement is stopped, animals will need to be euthanized for welfare reasons because facilities will rapidly become overcrowded. An investigation of the first FMD case requires trace-back of all animals that came onto a premises and may have introduced the disease, and trace-forward of all animals 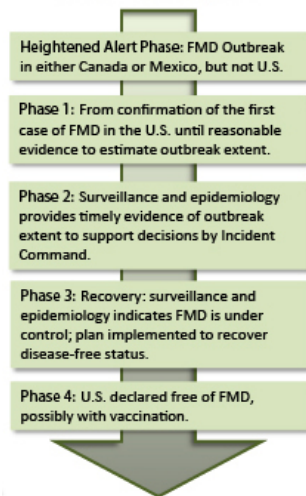that left the premises and may have spread the disease. Tracing is recommended by the World Organization for Animal Health (OIE) for all animals that entered or left the operation in the previous two incubation periods for the disease (the OIE recognized maximum incubation period for FMD is 14 days). The premises that are identified through tracing from the original premises need to be investigated and may need to be designated as contact or infected premises. A trace-back and trace-forward for 28 days will need to be conducted for each of these newly identified infected premises. This could be a monumental task, which quickly becomes impossible due to insufficient resources and lack of comprehensive, electronic real time animal ID and movement records.

3. POTENTIAL NEED FOR FMD VACCINE TO CONTROL AN OUTBREAK

The need for FMD vaccine depends on the phase and type of FMD outbreak and the number of strains of FMD virus involved in the outbreak. An explanation of the potential phases and types of FMD outbreak is found at: www.cfsph.iastate.edu/pdf/phases-and-types-of-an-fmd-outbreak.

Phases of FMD Outbreak in the U.S.   **Types of FMD Outbreaks during Phase 2**
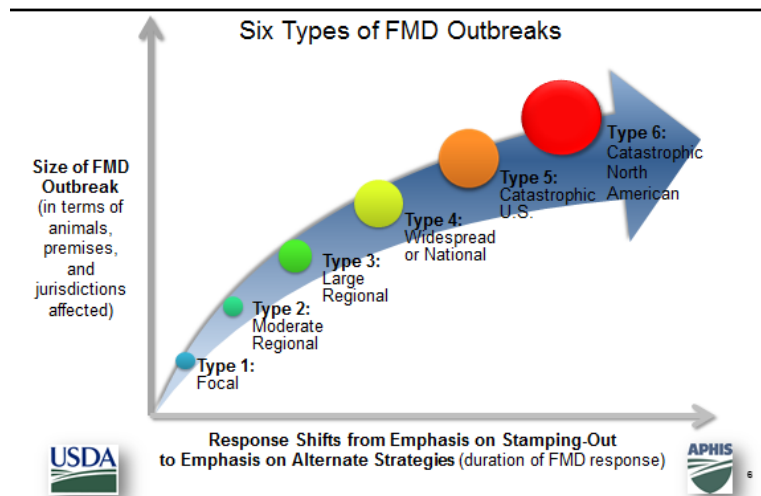
The amount of vaccine needed will depend on the phase and type of outbreak:

Phase 1: The period of time from the confirmation of the first FMD case in the United States until there is reasonable evidence to estimate the extent of the outbreak. The transition to Phase 2 should be accomplished as soon as possible, with a goal of less than 4 days (96 hours). No vaccine is used during this phase, however, vaccine could be ordered as soon as the preliminary vaccine matching to the outbreak strain is available.

Phase 2: Surveillance and epidemiology provides timely evidence of the extent of the outbreak to support planning and decision-making by Incident/Area Command. The strategy for vaccine use and amount of vaccine needed depends on the type of outbreak. The type of outbreak may change as the outbreak progresses.

Type of FMD Outbreak in Phase 2	Potential Vaccine Need
Type 1: Focal FMD outbreak	Stamping out. No vaccine will be needed.
Type 2: Moderate regional FMD outbreak: Ring vaccination-to-kill or vaccination-to-slaughter	Estimate that up to 10% of the U.S. livestock population may need to be vaccinated for six months
Type 3: Large regional FMD outbreak: Vaccination-to-slaughter and/or vaccination-to-live of a maximum of all susceptible domestic animals in the region. Discontinue vaccination 28 days after last case	Estimate that up to 30% of the U.S. livestock population may need to be vaccinated for one year
Type 4: Widespread or national FMD outbreak: Vaccination-to-slaughter or vaccination-to-live of a maximum of all susceptible domestic animals in the U.S. Continue vaccination after disease is under control to prevent re-emergence	Estimate that up to 100% of the U.S. livestock population may need to be vaccinated on an ongoing basis, to achieve OIE status of "FMD free with vaccination"
Type 5: Catastrophic U.S. outbreak: Insufficient vaccine and resources to effectively use vaccine as a control strategy. Transition to a long-term control and eradication program including vaccination to live of a maximum of all susceptible domestic animals in the US	As vaccine becomes available, vaccinate up to 100% of the U.S. livestock population on an ongoing basis. Vaccination may be necessary in certain populations for years

Phase 3: Surveillance and epidemiologic evidence indicates that the outbreak is under control and a plan is implemented to regain OIE FMD-free status (possibly with vaccination).

FMD free without vaccination will not require any additional vaccine (except in a stockpile in case FMD re-emerges).

FMD free with vaccination will require enough vaccine to vaccinate all designated susceptible animals on an ongoing basis (animals designated to be vaccinated will be decided by officials responsible for the control program).

Phase 4: The United States is declared free of FMD (possibly with vaccination). The USDA continues to work to convince trading partners to accept U.S. exports of animals and animal products.

FMD free without vaccination will not require any additional vaccine (except in a stockpile in case it re-emerges).

FMD free with vaccination will require enough vaccine to vaccinate all designated susceptible animals on an ongoing basis (animals designated to be vaccinated will be decided by officials responsible for the control program).

4. TRADITIONAL TOOLS AND APPROACHES USED TO CONTROL FMD OUTBREAKS

Table 1 lists the tools and approaches traditionally used to control an FMD outbreak in an FMD free country. In a large, rapidly spreading outbreak in the U.S. (type 3 or greater), the only tool from this list that currently is realistically available is biosecurity. It is the producer's responsibility to keep their herd from becoming infected with FMD, as with any other disease. Effective biosecurity would be especially difficult in an area with a high livestock density or large numbers of deer or feral swine. This demonstrates the importance of rapid availability of sufficient quantities of FMD vaccine as an essential tool to avoid catastrophic losses and to enable animal agriculture to return to a semblance of normal.

Table 1: Tools and approaches used to control FMD outbreaks

- Stop Movement
- Biosecurity
- Stamping Out
  - Depopulation of all infected herds and in-contact herds (within 24 hours or as soon as possible)
- Trace back/Trace forward
  - 28 days prior to outbreak
- Rapid Diagnostics
- Vaccination
  - Vaccinate to kill/Vaccinate to slaughter/Vaccinate to live

5. DETERMINATION OF PRIORITIES FOR VACCINE USE

In order to be fully prepared for the need to vaccinate in an FMD outbreak in the US, it is essential to assume that the outbreak may progress to a Type 5 outbreak very quickly and that it may involve multiple strains of FMD virus (due to an agroterrorism event or the accidental introduction of more than one serotype). This is a very challenging scenario. However, even in a type 5 FMD outbreak there will be animals and herds that are a higher priority for vaccination and other animals and herds that are a lower priority based on epidemiologic considerations, level of biosecurity, and other factors. **Therefore, in order to make the most effective use of available vaccines and maximize their impact in disease control, a working group of FMD vaccine experts and animal agriculture experts should be convened to establish priorities for the use of limited amounts of vaccine for a variety of scenarios.** The animal agriculture experts should be knowledgeable of production practices used by small and large producers for all of the FMD susceptible species.

Cattle are usually considered to be the highest priority for emergency vaccine use with most strains of FMDV. If the disease is under control in cattle, most FMDV strains should not persist in other species. For example, in the 2001 FMD outbreak in Uruguay, the outbreak was brought under control by rapid vaccination of all cattle in the country. To effectively induce immunity in the cattle population, all cattle

in the affected region should receive two doses of normal potency FMD vaccine one month apart, or a single dose of high potency FMD vaccine, as soon as possible. However, bringing the outbreak under control is just one of the major objectives of an FMD eradication program.

Another goal is to protect swine, sheep, and goat herds and other susceptible species from infection. In Uruguay in 2001 there was a relatively small swine population, which was not vaccinated. In high density swine production areas, vaccination of swine will be very important to reduce disease spread by swine because they exhale high concentrations of virus which may be spread by aerosol. Therefore, it would be desirable to also vaccinate those swine herds judged to be at risk of infection, as soon as possible. In a large outbreak (type 3 or larger) in which stamping out is discontinued, it is likely that FMD infected herds will be allowed to recover. Recovered herds should not need an initial dose of vaccine, but should be administered booster doses in approximately 6 months to ensure uniform immunity among animals in the herd.

6. REGULATORY CONSIDERATIONS FOR EMERGENCY USE OF FMD VACCINE

Prior to vaccination, vaccine use must be approved by the USDA Center for Veterinary Biologics or allowed under a USDA exemption. Seven possible approaches for vaccine approval are listed below.

(The text in italics in this section is adapted from: R.E. Hill, P.L. Foley, M.Y. Carr, L.A. Elsken, D.M. Gatewood, L.R. Ludemann, and L.A. Wilbur; "Regulatory Considerations for Emergency Use of Non-USDA Licensed Vaccines in the United States" in F. Brown and J.A. Roth, (eds) Vaccines and Diagnostics for List A and Emerging Animal Diseases. Dev. Biol. Basel, Karger, 2003, Vol 114, pp 31-52.)

The Virus-Serum-Toxin Act of 1913 (21 U.S. Code 151-159) provides the legal basis for the regulation of veterinary biologicals in the United States; the United States Department of Agriculture's Center for Veterinary Biologics (CVB) has the regulatory authority for the issuance of licenses and permits for such products. Administrative regulations and standards appear in Title 9, Code of Federal Regulations, Parts 101-118, with additional program guidance found in CVB Notices, Veterinary Services Memoranda, General Licensing Considerations, and other guidance documents. Under the standard licensing process, this spectrum of evaluation includes complete characterization and identification of seed material and ingredients, laboratory and host animal safety and efficacy studies, stability studies, and post-licensing monitoring of field performance. This comprehensive evaluation may not be possible during the emergence of a new animal disease. While there are no specific regulations addressing the licensing standards of products for an emerging animal disease, there are mechanisms that allow for the availability of products in an emergency animal health situation. There are 7 methods for achieving vaccine approval. The first method is the conventional licensing process for products, utilized under normal circumstances; the remaining 6 include various options for use under emergency or emerging animal disease conditions

6.1 Conventional product licensing requirements (9 CFR Parts 101-118)

Licensing of a conventional vaccine requires submission of data on purity, potency, safety, and efficacy. The vast majority of animal vaccines used in the U.S. are licensed under these requirements. This process provides the greatest assurance of purity, potency, safety, and efficacy of veterinary vaccines and provides the greatest assurance to livestock producers that vaccines are safe and effective.

Ideally, vaccines for FMD should be licensed to meet the conventional product licensing requirements. However, this is a lengthy and relatively expensive process. Manufacturers do not have a sufficient incentive to meet these licensing requirements since there is not a market for FMD vaccines in the U.S. prior to an outbreak. In addition, it is currently illegal to have the FMD virus on the U.S. mainland; even for vaccine production purposes (the Secretary of Agriculture has some discretion to make exceptions as described in 21 USC 113a). During an outbreak, this process would not be able to generate a new vaccine in time to aid eradication efforts, although it might yield a vaccine for longer-term control of a virus that became established in the U.S. There are no FMD vaccines conventionally licensed in the U.S.

6.2 Experimental product approvals (9 CFR Part 103.3)

The approval process for conventional vaccine licensing allows limited amounts of experimental vaccine to be tested in animals under controlled conditions.

This approval process does not provide a viable approach for rapid availability of large volumes of vaccine.

6.3 Autogenous product licenses (9 CFR Part 113.113)

All autogenous vaccines are prepared from cultures of microorganisms that have been inactivated and are non-toxic. The microorganisms used as seed must be isolated from sick or dead animals in the herd of origin. They are to be used only by or under the direction of a veterinarian with a veterinary-client-patient relationship. They are produced under a USDA APHIS CVB approved outline of production in an approved facility. However, autogenous vaccines cannot be made from Select Agents.

FMD virus is a select agent. Therefore, the autogenous product license cannot be used to prepare FMD vaccines, unless the FMD virus is removed from the select agent list after an outbreak. Adapting a field strain of FMD virus to manufacturing growth conditions typically requires several months.

6.4 Conditional product licenses (9 CFR Part 104)

Conditional licenses are authorized under very specialized circumstances to meet an emergency condition, limited market, local situation, or other special circumstance. Licenses are issued under an expedited procedure which assures purity and safety, and a reasonable expectation of efficacy of the

products involved. Preparation of products under a conditional license must be in compliance with all applicable regulations and standards and may be restricted.

The conditional license approval seems to be a viable process for licensing of FMD vaccines for emergency use. Demonstrating the purity and safety of FMD vaccines is very important for assuring the public that products from vaccinated animals are safe for consumption (e.g., assurance that all vaccine components are free of bovine spongiform encephalopathy prions). Demonstrating FMD vaccine efficacy against the outbreak strain(s) is a time consuming process. However, FMD vaccines that are well matched to the outbreak strain and have high potency have a reasonable expectation of efficacy. The Department of Homeland Security (DHS) has provided funding which has enabled one human adenovirus-vectored FMD serotype A24 vaccine to meet the requirements for a conditional license (other strains are under development). Stockpiles of this vaccine are not immediately available in sufficient quantity for rapid use in controlling an outbreak. The manufacturer would need to increase production once a need became apparent. It would likely require several weeks to begin to produce vaccine and many months to produce sufficient vaccine to meet the potential need.

6.5 U.S. Veterinary Biological Product Permit for Distribution and Sale (9 CFR Part 104)

This permit is for vaccines manufactured outside of the U.S. These vaccines must meet the same purity, potency, safety, and efficacy requirements as vaccines issued a conventional product license (#1 above). Permits for importation, distribution, and sale may be issued to persons who reside in the U.S., or operate a business establishment within the U.S.

The Department of Homeland Security has provided funding to enable one conventional inactivated FMD vaccine to be permitted for distribution and sale in the U.S. under the supervision and control of USDA, APHIS, Veterinary Services as part of an official USDA animal disease control program. The vaccine is a quadrivalent FMD vaccine (strains A24 Cruzeiro, A2001 Argentina, C3 Indaial, and O1 Campos) produced by Biogenesis Bago in Argentina. Stockpiles of this vaccine are not immediately available in sufficient quantity for rapid use in controlling an outbreak. The manufacturer would need to increase production after a need became apparent. Several weeks would be required to begin to produce vaccine and several months (or years) to produce sufficient vaccine to meet the potential need. Possible options are to enter into contracts with companies that receive permits for a vendor-managed inventory of finished vaccine or vaccine antigen concentrate dedicated to the U.S. and/or for surge capacity production in the event of an outbreak. Without a contract, the manufacturer will likely have committed all of its production to current customers and may not be prepared for surge capacity production.

6.6 Antigen "Vaccine" bank

No specific regulations exist for the creation and maintenance of a vaccine or seed bank. Where the potential need for rapidly available vaccine exists, a bank can be used to store relevant strains as inactivated antigen concentrates and live master seeds. Bank components are pretested and approved. It is necessary to reevaluate the strains within the bank periodically for antigenic relevance as well as degradation of product during storage.

The North American Foot-and-Mouth Disease Vaccine Bank is an antigen bank, containing VAC, which is shared by the U.S., Canada, and Mexico, but it does not contain sufficient FMD vaccine antigen for even one livestock dense state. The National Veterinary Stockpile could also serve as a vaccine bank for FMD vaccines for emergency use in the U.S.

6.7 USDA exemption (9 CFR Part 106.1)

Under very specialized circumstances, biological products may be exempted from one or more of the requirements of the 9 CFR Parts 101-118. These circumstances are warranted if products will be used by the USDA or under the supervision or control of the USDA in the prevention, control or eradication of animal diseases in connection with (a) an official USDA program; or (b) an emergency animal disease situation, or (c) a USDA experimental use of the product.

This exemption could be used to import commercial vaccines, produced in other countries, which have not been evaluated by CVB to meet the requirements for importation, sale and distribution described in 5 above. It would be very important to ensure that the imported vaccines were produced with irradiated serum in the growth medium and that the serum was derived from bovine spongiform encephalopathy free animals.

This exemption could also be used to allow U.S. manufacturers to produce vaccine for emergency use which has not completed the full conventional or conditional licensure process. Allowing U.S. vaccine manufacturers to produce killed adjuvanted FMD vaccine using a USDA approved FMD master seed strain, master cell line and outline of production provided by the NVS could significantly expand the number of doses of vaccine available within several weeks in the U.S. The outline of production may need to be adapted to the manufacturer's equipment and capabilities. The manufacturer would need to have excellent biosecurity standard operating procedures. Potency and efficacy testing of these emergency vaccines could be based on vaccination trials followed by evaluation of serum neutralizing antibody titers against the outbreak strain. This method of potency and efficacy testing is not as rigorous as the testing required of fully licensed vaccines, but could be completed in a few weeks in order to make vaccine available. A concern is that vaccines produced without careful quality control to remove nonstructural proteins (NSPs) from the vaccine, and validation of the absence of antibody responses to NSPs would not be DIVA compatible. Those vaccines could not be used to vaccinate animals intended to live for an extended time, but could be used to vaccinate feedlot cattle or swine intended for slaughter, without compromising the ability of the U.S. to eventually become free of FMD with vaccination.

7. VACCINES APPROVED FOR INCLUSION IN THE NVS AND FOR EMERGENCY USE IN THE U.S.

All stakeholders need to be assured that FMD emergency use vaccines are safe for the vaccinated animals and for the public consuming products from vaccinated animals. Ideally, the FMD vaccine(s) should be evaluated by the USDA Center for Veterinary Biologics and found to be pure, potent, safe, and effective, that is, they should meet the same requirements as vaccines for endemic diseases that are USDA licensed. A recommendation to be considered is that all FMD vaccines produced in the original E.U. member states (Maastricht Treaty; member states prior to 1994) that have either previously obtained EMA CVMP marketing authorization at the national level in one or more original E.U. member states, or single marketing authorization using the multi-strain dossier approach for use across all E.U. Member States could be considered to be safe and effective and pre-approved for emergency use in the U.S. These vaccines could be granted a USDA Exemption (9 CFR Part 106.1) from one or more of the requirements of the 9 CFR Parts 101-118 for emergency use in the U.S. The U.S. and E.U. systems for evaluating vaccine safety and efficacy have been extensively compared through the Veterinary International Committee on Harmonization (VICH). The two systems are different, but each assures vaccine safety and efficacy. This would allow FMD vaccines licensed in the E.U. which meet the criteria above to be included in the NVS as either vaccine antigen concentrates, or finished vaccine in a Vendor Managed Inventory arrangement, and could allow pre-approval for importation, sale, and distribution of finished vaccine in an emergency.

8. VACCINE STRAINS RECOMMENDED FOR NATIONAL ANTIGEN BANKS

In September 2013 the World Reference Laboratory for Foot and Mouth Disease at the Pirbright Institute in Pirbright, UK recommended that national antigen banks for FMD maintain 23 strains of FMD virus (as live master seeds and inactivated antigen concentrates). The following strains (http://www.wrlfmd.org/ref_labs/fmd_ref_lab_reports.htm) are included in that list (not in order of importance within priority).

High Priority:
- O Manisa
- O PanAsia-2
- O BFS or Campos
- A-Iran-05 (or A TUR 06)
- A24 Cruzeiro
- A22 Iraq
- Asia 1 Shamir
- SAT 2 Saudi Arabia (or equivalent i.e. SAT 2 Eritrea)

Medium Priority:
- A Argentina 2001
- A Eritrea
- A Malaysia 97 (or Thai equivalent such as A/NPT/TAI/86)
- O Taiwan 97 (pig-adapted strain or Philippine equivalent)
- SAT 1 South Africa
- SAT 2 Zimbabwe

Low Priority:
- A Iran 96
- A Iran 99
- A Iran 87 or A Saudi Arabia 23/86 (or equivalent)
- A 15 Bangkok related strain
- A87 Argentina related strain
- SAT 1 Kenya
- SAT 2 Kenya
- SAT 3 Zimbabwe
- C Noville

9. IMPORTANCE OF EXPEDITED PROCESS FOR SWITCHING VACCINE SEROTYPES, TOPOTYPES IN A PROVEN PLATFORM.

Each FMD vaccine that is conventionally licensed, conditionally licensed, or permitted for distribution and sale covers one or a few specific strains of FMD virus. Currently, each new strain is treated as a new vaccine and requires the same testing and data as the originally licensed strain. Since there are 23 strains recommended to be available in national FMD stockpiles, having all 23 strains in adequate amounts in the stockpile for the duration of the outbreak represents an enormous expense. Some of the newer vaccine technologies described in the Appendix to this white paper represent a vaccine backbone that can quickly be modified by substituting the genes for the capsid proteins from

the outbreak strain to produce a vaccine to protect against the outbreak strain(s) (eg. hAd5 vectored vaccines, leaderless killed FMD-LL3B3D vaccines, Alphavirus replicon particle vaccines, plasmid DNA vaccines, and baculovirus produced vaccines). Inserting the capsid genes of the new strain in a killed or non-replicating vaccine is not likely to change the safety profile of the vaccine. The efficacy would still need to be proven in the species that are intended for vaccination, but there is a reasonable expectation that the newly derived vaccine would be effective. Such a vaccine could be issued a conditional license in order to make it more rapidly available while the efficacy testing is conducted. The USDA issued Veterinary Services Memorandum number 800.213 in August 2013: "Guidelines for Obtaining a Conditional Veterinary Biologics License for Production Platform Derived, Recombinant, Non-replicating, Nonviable Constructs". This memorandum provides guidance to licensees, permittees, and applicants, regarding the licensure of Production Platforms based on recombinant technology and resulting in non-replicating, nonviable biological products. This new memorandum should facilitate the rapid adaptation to manufacturing of some new generation vaccines to include new outbreak strains.

10. DESIRABLE CHARACTERISTICS OF VACCINES AND OF VACCINE PRODUCTION THAT COULD MEET THE NEED IN A LARGE FMD OUTBREAK IN THE US:

The following is a list of desirable (but not necessarily essential) characteristics for FMD vaccines and vaccine production technology that could help meet the need for rapid production of large volumes of FMD vaccine, to aid in the control of an FMD outbreak in the U.S.

- Rapid onset of immunity

- Long duration of immunity

- One dose does not interfere with booster doses or other vaccines used in livestock

- Effective in presence of maternal antibody

- Broad protection within serotypes

- Safe for manufacturing under BL2 conditions in a disease free country

- Vaccine organism is not a select agent

- Rapid conversion of manufacturing to outbreak strain

- Companion diagnostic test to detect infection in vaccinated animals (DIVA)

- Capability to meet 9 CFR regulatory requirements for purity, potency, safety, and efficacy

- Capability to meet requirements for cost effective manufacturing and all proprietary (patent) rights to vaccine antigen, vectors, and/or adjuvants

- Safe for use in food producing animals with no, or reasonably short, withdrawal time for animal products for human consumption

- Not hazardous for humans accidentally exposed to the vaccine

- Safety and efficacy demonstrated in multiple species (especially cattle and swine)

- Safety and efficacy under field conditions in an outbreak and in an endemic country are established

- Safe and effective when delivered orally in baits for feral swine or deer

- Compatible with World Organization for Animal Health (OIE) requirements for safety and efficacy of vaccines for international trade

- Desirable characteristics of vaccines for emergency use vaccine or VAC stockpiles

  o Ongoing manufacture and sale in endemic countries which enables indefinite delivery/indefinite quantity (IDIQ) contracts for just in time delivery

  o Stable when stored as bulk vaccine antigen concentrate

  o Long self-life and stability of finished vaccine

  o Heat stable (e.g., considerations of need for cold chain)

  o Rapid scale up and manufacture in the U.S. in an emergency

The currently available vaccines and the FMD vaccines under development are reviewed in the Appendix to this document for their ability to meet these characteristics. No single technology can meet all of the desirable characteristics. Each technology has advantages and disadvantages, and some desirable characteristics (e.g., absence of interference with maternal antibodies) may be difficult to meet with any vaccine. Currently, there are no short-term prospects for oral FMD vaccines for wildlife and feral animals.

11. APPROACHES AND GOALS FOR ASSURING SURGE CAPACITY FOR FMD VACCINE FOR THE U.S.

A combination of approaches can be used to assure surge capacity for FMD vaccines.

1. **Immediate Availability:** Finished vaccine held in vendor-managed-inventory and ready for shipment within 24 hours.

   a. Sufficient doses (5 to 10 million?) of finished vaccine held in vendor-managed inventory at USDA licensed/permitted or approved E.U. licensed FMD manufacturers for all strains of FMD virus considered to be a threat to the U.S. Finished vaccine should be able to be shipped to the U.S. within 24 hours of request for shipment. The amount of finished vaccine needed depends on the time required for vaccine antigen concentrate (#2 below) to be formulated and shipped to the U.S.

2. **Short-Term Availability:** Vaccine antigen concentrate (VAC) held in vendor-managed-inventory ready to be formulated into finished vaccine and shipped to the U.S.

a. Sufficient doses (15 to 50 million?) of FMD VAC held in vendor-managed-inventory at USDA licensed/permitted or approved E.U. licensed FMD manufacturers for all strains of FMD virus considered to be a threat to the U.S. The amount of VAC needed depends on the time required for the vaccine to become available, if vaccine production is initiated at the beginning of the outbreak for the outbreak strain(s).

3. **Long-Term Availability:** Vaccine production initiated at the beginning of the outbreak for the specific outbreak strain(s) of FMD virus.

a. Sufficient doses to keep up with the demand for FMD vaccine during the outbreak and perhaps for a vaccinate-to-live program after the outbreak is under control (up to 160 million doses every 6 months if all cattle and swine in the U.S. are to be vaccinated).

It is likely to require many years to achieve all the goals above. A plan is needed to ensure step-wise progression over several years to achieve these goals in the most efficient and cost effective way possible. The initial focus should be on vaccines for the highest priority strains of FMD virus recommended by the FMD World Reference Laboratory. The vendor-managed-inventory approach appears to be the most cost effective method to ensure rapid FMD vaccine availability in the immediate future. Indefinite delivery indefinite quantity (IDIQ) contracts could be entered into with FMD manufacturers who are licensed by either the USDA Center for Veterinary Biologics (currently Merial and Biogenesis Bago) or by the European Union (as described above) (Currently MSD Animal Health (Merck) and Merial).

Vendor-managed-inventory contracts for finished FMD vaccine: The manufacturer would be paid to maintain a prescribed number of vaccine doses in its inventory at all times for immediate shipment to the U.S. The manufacturer rotates the stock by holding the most recently manufactured doses and by selling doses from the inventory as new doses are added. There would be a cost associated with maintaining inventory ready for shipment and an additional cost to purchase the vaccine if needed. This option only exists for FMD vaccines that are routinely being manufactured and sold by biologics companies.

Vendor-managed-inventory contracts for VAC: The manufacturer would be paid to produce and store VAC in their facilities. Before the VAC expires, it would be replaced by new VAC, then formulated into finished vaccine and sold by the company to their usual customers. This would require the manufacturer to alter its production practices by producing the VAC, then holding it for multiple years and monitoring it for quality assurance before formulating it into vaccine. This option only exists for FMD vaccines that are routinely being manufactured and sold by biologics companies.

Vaccine production at the beginning of the outbreak will require that the manufacturers have the capacity to rapidly scale up production, and to also meet their obligations for supplying vaccine to their regular customers. Ideally, vaccine production would occur in the U.S., to avoid concerns that regulatory authorities in other countries may have with expedited approval of vaccine for export and with their

priority for meeting FMD vaccine needs within their own countries. The manufacturer(s) should maintain approved master seed virus for multiple serotypes of FMD virus and approved master cell lines ready to grow the virus. The time required to produce vaccine at the beginning of the outbreak is likely to vary with the manufacturer and the technology used. It will be important to obtain information from various manufacturers on the vaccine strains they are prepared to manufacture, number of doses they can produce and the time to deliver the finished vaccine from the moment that an order is placed at the beginning of an outbreak. This confidential business information, along with estimated costs, will be essential for developing a detailed plan to meet the emergency need for FMD vaccine in the U.S.

12. DECISION MAKING ON BEST APPROACH TO ENSURING FMD VACCINE AVAILABILITY

This white paper and the Appendix include publically available and company supplied information which is important for decision making on the best approaches to ensuring FMD vaccine availability. However, additional information is needed (which in many cases is confidential business information) from the companies with the vaccine technology. This information includes:

- Cost estimates for:

    - Vendor-managed-inventory of 5 to 10 million doses of finished vaccine ready for shipment

    - Vendor-managed-inventory of vaccine antigen concentrate (VAC) ready to be formulated into finished vaccine

    - Vaccine production initiated at the beginning of the outbreak

- FMD vaccine strains which are routinely produced and sold and which would be candidates for vendor-managed-inventory of finished vaccine and/or VAC and for production at the beginning of the outbreak.

- Time required to formulate VAC into finished vaccine and for quality assurance (QA) testing for vaccine release

- Time required and capacity for vaccine production initiated at the beginning of the outbreak

- Projected time to licensure for novel vaccine technologies under development

A working group of experts capable of evaluating existing and new technology FMD vaccines under development should be established to determine the technologies that can best meet the needs for emergency response vaccination in the US. The biologics companies developing the new technology vaccines have received and reviewed the section of the Appendix to this document pertaining to their vaccine technology. In some cases, they have added information cited as personal communication. All of the information in this manuscript related to these vaccines is approved for public release by the

companies involved. The working group could enter into confidentiality agreements with the biologics companies, to have access to confidential business information which can inform the recommendations for incorporating vaccines under development into the surge capacity plan. The working group could also seek confidential business information from manufacturers on the cost of the various approaches listed above, in order to make recommendations on the most cost effective approach to meeting the surge capacity need for FMD vaccine. Current manufacturers of FMD vaccines and companies developing new technology vaccines will likely have novel suggestions for how they could help meet this need, and could be invited to recommend innovative approaches. This information would need to be kept confidential during the plan development phase. The procurement of vaccines for the NVS would need to be through USDA contracting procedures. The working group would need advice from USDA contracting officials to ensure that recommendations are consistent with USDA contracting policies and procedures. The working group could make recommendations to the industry stakeholder committee on optimal approaches to meeting the surge capacity needs, based on the confidential business information they have received, without divulging the information. Any contracts for supplying FMD vaccine for the NVS would need to be competitively awarded through standard U.S. government processes.

13. SUMMARY OF POTENTIAL SOLUTIONS TO PROVIDE ADEQUATE FMD VACCINE TO CONTROL A LARGE OUTBREAK OF FMD IN THE U.S.

1) A combination of approaches can be used to assure surge capacity for FMD vaccines.

   a. Immediate Availability: Finished vaccine held in vendor-managed-inventory and ready for shipment within 24 hours.

      i. Enter into vendor-managed-inventory contracts with international manufacturers of FMD vaccines, for rapid delivery of multiple serotypes of finished vaccines which have been permitted for importation and sale into the U.S. All FMD vaccines that are licensed or permitted by USDA CVB for use in the U.S. and all FMD vaccines produced in the original E.U. member states (Maastricht Treaty; member states prior to 1994) that have either previously obtained EMA CVMP marketing authorization at the national level in one or more original E.U. member states, or single marketing authorization using the multi-strain dossier approach for use across all E.U. Member States could be considered to be safe and effective and pre-approved for emergency use in the U.S. Contracts should be developed to provide enough vaccine to supply the U.S. during approximately the first two weeks of an outbreak.

   b. Short-Term Availability: Vaccine antigen concentrate (VAC) held in vendor-managed-inventory ready to be formulated into finished vaccine and shipped to the U.S.

<ol type="i" start="1">
Stockpile multiple strains of vaccine antigen concentrate (VAC) in the National Veterinary Stockpile (NVS). Enough VAC should be available for the period between depletion of the finished vaccine and availability of large amounts of vaccine available from production initiated at the beginning of the outbreak. The VAC should be held at the approved manufacturer (see a.1. above) and the contract could support a rotating inventory by formulating the VAC into finished vaccine for sale and replacing it on a regular basis.

<ol type="a" start="3">
<li value="3">Long-Term Availability: Vaccine production initiated at the beginning of the outbreak for the specific outbreak strain(s) of FMD virus.
<ol type="i">
Enter into contracts with international manufacturers of FMD vaccines for surge capacity production of commercially available USDA-permitted or E.U. licensed (see a.1. above) FMD vaccines.
Seek USDA licensure of new technology FMD vaccines that could be safely manufactured in the U.S. and which are based on a platform that allows capsid types from various strains to be inserted into the vaccine platform. These would then be candidates for vendor managed inventory of finished vaccine and of VAC. Ensure that U.S. manufacturers have the surge capacity to rapidly produce finished vaccine at the beginning of an outbreak.

<ol start="2">
Ensure that all FMD vaccines used are DIVA compatible (unless animals are intended for slaughter). Ensure that sufficient reagents and/or finished kits for DIVA testing will be available for the recovery phase of the FMD outbreak and sufficient NAHLN labs have been equipped, trained and proficiency tested to conduct this assay.

Develop and adopt technology and scalable information technology for identifying and tracking all vaccinated animals and diagnostic testing results.

Develop interferon or other antiviral biotherapeutic products for inducing rapid and medium term resistance (1 day to 14 days) to FMD infection (a long term goal).

Form a standing advisory committee with expertise in FMD vaccines, production agriculture, and emergency response to make recommendations on optimal use of vaccine as the outbreak unfolds.

Secure funds to enable meeting the surge capacity need for FMD vaccines mandated in HSPD 9 (estimated at $150 million/year for 5 years to help protect animal industries with approximately $100 billion dollars in cash receipts per year).

As part of this effort, DHS S&T should conduct a classified Biological Threat Risk Assessment (BTRA) in collaboration with the USDA (APHIS and ARS), the Department of Commerce, and the Office of National Intelligence. Such a BTRA shall include the size and economic scope of the livestock industry at risk; the potential sources of virulent FMD virus; the potential

routes of incursion into the U.S. (both from natural or intentional introduction); the potential Foreign Terrorist Organizations (FTOs) with capability and interest to utilize FMD virus; an assessment of the ease of obtaining, transporting, and delivering virulent FMD virus; and the impact to the U.S. economy of an FMD outbreak in the U.S. (whether it be natural or intentional).

7) Convene a stakeholder community working group of experts capable of evaluating existing and new technology FMD vaccines under development, to determine the technologies which can best meet the needs for emergency response vaccination in the US. The working group could enter into confidentiality agreements with biologics companies, to have access to confidential business information which can inform the recommendations for incorporating existing and new vaccines into the surge capacity plan.

8) Conduct research into alternative delivery methods for vaccines which have been shown in cattle and swine to significantly reduce the antigenic mass required in each dose of vaccine, thus enabling existing or future VAC to be formulated into significantly more doses of vaccine.

Acknowledgements

Appendix A

Comparison of Foot and Mouth Disease Vaccines and an Evaluation of Antiviral Prophylaxis Methods against FMDV

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Appendix A contains two sections. The first, entitled Comparison of FMD Vaccines: Conventional Inactivated Vaccines, Leaderless LL3B3D Inactivated Vaccines, hAd5 Vectored Vaccines, Alphavirus Replicon Vaccines and Plasmid DNA Vaccines, provides a summary of each vaccine type, based on the desirable characteristic for FMD vaccines. An initial description of each type of vaccine is followed by a brief description of its ability to meet each of the characteristics. Some of the information described is based on published sources; the remainder has been supplied by vaccine company personnel and other sources. In many cases, the information is still preliminary and partial, as the vaccines are still in development.

The second section is a brief literature review, entitled *Antiviral Prophylaxis for the Control of Foot and Mouth Disease*. It summarizes a number of approaches that might be used to help control FMDV during the initial stages of an outbreak, while vaccine-induced immunity is still developing.

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Section I: Comparison of FMD Vaccines: Conventional Inactivated Vaccines, Leaderless LL3B3D Inactivated Vaccines, hAd5 Vectored Vaccines, Alphavirus Replicon Vaccines and Plasmid DNA Vaccines

1. STRUCTURE OF THE FMDV VIRION AND FUNCTIONS OF THE VIRAL PROTEINS

FMDV is a member of the genus *Aphthovirus* in the family Picornaviridae. The FMDV virion contains a positive sense, single stranded RNA genome inside an icosahedral capsid. The capsid consists of 60 copies of each of four proteins: 1A (also called VP4), 1B (VP2), 1C (VP3) and 1D (VP1).[1] Replication takes place in the cytoplasm of the host cell. The viral genome is initially translated into a single polyprotein, which is cleaved by viral proteases into both capsid proteins and non-structural proteins (NSPs).[1] The latter are involved in the replication of FMDV genome and cleavage of its protein products, as well as the inhibition or alteration of certain host cell functions.[1] The primary cleavage products after translation are 1) the N-terminal leader protease L^{pro}; 2) P1-2A, a precursor protein that is cleaved to form the capsid proteins and the nonstructural protein 2A; 3) the nonstructural protein 2BC; and 4) the precursor protein P3, which is cleaved to become the NSPs 3A, 3B, 3C and 3D.[1] The 3C protease cleaves P1 to form three products: 1AB (VP0), 1C (VP3) and 1D (VP1). The 1AB product is later cleaved to form 1A (VP4) and 1B (VP2) at the stage when FMDV RNA is encapsidated.[1] Many of the viral-vectored FMD vaccines in development incorporate the coding sequence for the P1-2A region of the FMD genome. Some also include coding sequences for some NSPs.

Table 1: Selected functions of FMDV Nonstructural Proteins

NSP	Role in Virus Replication	Importance in vaccination
L (Leader protease)	Involved in cleavage of viral polypeptide; also cleaves host proteins to reduce translation of host mRNAs[1]; Not necessary for capsid assembly[1-3]	Modified in leaderless FMD vaccine

2A	Protease, involved in cleavage of FMDV polypeptide[1]; Not necessary for capsid assembly[1-3]	Included in hAd5-vectored FMD vaccines
2B	Alteration in cell functions, including changes in endoplasmic reticulum and Golgi; involved in RNA amplification.[1] Proteins 2B, 2C, and 3A have been implicated in membrane rearrangements that produce the cytoplasmic vesicles where FMDV replicates.[4]	Full length gene included in a recent version of the hAd5-vectored A_{24} Cruzeiro vaccine, resulted in improved efficacy
2C	May be involved in host cell membrane changes that eventually allow release of virions; directs replication complexes to cell membrane; may be involved in virus encapsidation.[1] Proteins 2B, 2C, and 3A have been implicated in membrane rearrangements that produce the cytoplasmic vesicles where FMDV replicates.[4]	
3A	Inhibits MHC class I expression; involved in changes in cell functions.[1] Changes in 3A might influence outcome of infection, result in changes in host range.[1] Proteins 2B, 2C, and 3A have been implicated in membrane rearrangements that produce the cytoplasmic vesicles where FMDV replicates.[4]	Most commercial NSP (ELISA) tests are based on the 3AB or 3ABC protein, and are used as serological DIVA tests with conventional inactivated vaccines and some experimental FMD vaccines.
3B	Involved in synthesis of FMDV RNA; stimulates 3CD autocleavage (as 3AB).[1]	Modified to give DIVA capability in the leaderless LL3B3D FMD vaccine; Test based on this protein is in development as a serological DIVA test for hAd5-vectored vaccine. Most commercial NSP (ELISA) tests are based on the 3AB or 3ABC protein, and are used as serological DIVA tests with conventional inactivated

		vaccines and some experimental FMD vaccines. The 3B protein is the target for the competitive monoclonal antibodies used in most commercial cELISA tests.
3C	Protease required for capsid assembly; involved in host protein synthesis shutoff and transcription inhibition; RNA binding in RNA replication.[1,3]	Included in hAd5-vectored FMD vaccines to produce empty capsids from the P1-2A precursor.[5-7 cited in 8] Most commercial NSP (ELISA) tests are based on the 3AB or 3ABC protein, and are used as serological DIVA tests with conventional inactivated vaccines and some experimental FMD vaccines.
3D	Viral RNA-dependent RNA polymerase (synthesis of FMDV RNA); produces both positive sense and negative sense RNA.[1]	Often incorporated into the capsid and cannot be purified from conventional inactivated vaccines.[1,9]; Modified to give DIVA capability in the leaderless FMD-LL3B3D killed vaccine

2. VIRUS REPLICATION AND IMMUNE RESPONSES AFTER INFECTION

Initially, FMDV replication is thought to occur locally (e.g., in the nasopharynx of cattle infected via aerosols).[10] Localized replication is followed by dissemination of the virus in the blood to secondary replication sites.[2] Viremia usually lasts 2-3 days, and ends when circulating antibodies appear,[11;12 cited in 2] with viruses in the blood becoming undetectable as soon as 3-5 days after the first signs of illness.[11] Absence of detectable viremia has been used in some studies to support claims of vaccine efficacy in

preventing virus dissemination and disseminated disease. However, generalized lesions were reported in vaccinated animals that had no evidence of viremia, in one experiment.[13] Possible explanations are that the period of viremia was greatly reduced but not eliminated, and occurred before the first sampling,[13] or that the methods used are not always sensitive enough and/ or sampling is done too infrequently to always detect virus in the blood.

Humoral immune responses, with the production of neutralizing antibodies, are generally correlated with recovery from infection with FMDV and resistance to reinfection.[14-18] In cattle, the antibody response to FMDV was reported to be partly T_H-cell independent, and recovery was unaffected in CD4+ T cell depleted animals.[19] Evidence also exists for T_H-cell dependent responses.[18] Cell-mediated immune responses (CMI) have been reported in FMDV infected animals, although the role of this form of immunity is still under investigation.[15,18,20] One observation frequently cited for the potential importance of CMI, at least under some conditions, is that neutralizing antibodies are not invariably correlated with immunity to FMDV: vaccinated animals with moderate to high neutralizing antibody titers are not always protected from challenge, and animals with low or absent titers are sometimes protected.[14,15] It has been noted that the high variability in challenge experiments, which typically use only a few animals in each group, might also result in an apparent lack of correlation between antibody titers and protection from challenge.[18] More robust evidence is provided by an experiment which directly measured CTL responses in pigs, and found that the induction of such responses resulted in delayed clinical signs and reduced levels of viremia.[20] Cytokines may be involved in immunity to FMD, and interferons can inhibit the replication of FMDV in cell cultures.[21,22] One study reported that the levels of interferon γ, apparently produced mainly by CD4+ T cells, correlated with vaccine-induced immunity in cattle.[15] Mucosal immune responses, with the production of IgA, have been reported after FMDV infection, and might also play a role in protection.[14,18]

2.1 FMDV serotypes, strains and cross-protection

There are seven major serotypes of FMDV (O, A, C, Asia-1, SAT-1, SAT-2 and SAT-3), and most sources state that there is no significant cross-protection between serotypes.[23] However, there have been a few reports of cases where cattle infected by one serotype developed milder clinical signs or remained asymptomatic when they later were infected by other serotypes.[24 cited in 18] In these cases, the level of cross-neutralizing antibodies paralleled the protection.[18] One possible explanation is that immune responses to NSPs, which are highly conserved, might provide some protection after

repeated infection.[18] There is also some limited evidence that epitopes recognized by CD8+ T cells may be highly conserved between FMDV serotypes.[25]

Within a serotype, protection between strains varies with their antigenic similarity. Some serotypes are more variable than others, and it is possible that factors such as the structural needs of the virus capsid constrain the evolution of new strains.[26 cited in 17] As an example, the predicted rate of evolution of the Asia 1 serotype (10^{-2} substitutions per nucleotide position per year) would be expected to result in significant variability, but only one topotype was considered to exist as recently as 2012.[17,27] While new Asia 1 variants, which are poorly matched with the Asia 1 Shamir vaccine strain, have been recognized during recent outbreaks, a high potency, emergency vaccine was able to protect all cattle challenged with one of these field isolates.[27] Although the situation may well change in the future, only one strain of Asia 1 was still recommended for immunization and vaccine banking in the 2012 FAO World Reference Laboratory and OIE Reference Laboratory for FMD (WRL FMD) annual report.[27] One strain is also recommended for serotype C, which has also become very rare, and has not been reported since 2004.[27] Serotype O is genetically diverse, but antigenically restricted, and animals can be protected from most currently circulating viruses with a small number of vaccine strains.[27,28] In contrast, serotype A and the SAT viruses are genetically and antigenically diverse, and multiple vaccines are needed, as they must closely match the outbreak strain.[17,27,28] SAT viruses have been limited in their geographic distribution, and seem to persist long-term only in Africa, with periodic incursions into the Middle East.[29,30] However, these viruses could become a very serious problem if they became more widely distributed.[17] SAT-2 viruses, in particular, have higher sequence variability in the 1D (VP1) capsid protein than serotypes A, O and C.[31 cited in 17]

2.2 Differentiation of vaccinated from infected animals based on serological responses to FMDV proteins

No vaccine can be expected to provide sterile immunity in all animals, given the influence of factors such as age, genetic background, nutritional adequacy, concurrent illnesses and immunosuppression (e.g., by shipping stress) on the host immune response. Other variables that are incompletely controlled outside the laboratory, including vaccine administration (e.g., breaks in the cold chain or administration of less than a full dose), as well as exposure to large amounts of the pathogen, can also result in suboptimal responses. Therefore, there is a need to identify animals or herds that become infected after vaccination.

Serological tests are an important component in cost-effective surveillance. During virus replication, immune responses develop to both structural proteins and NSPs. The antibody titer to each protein is influenced by exposure. Infected animals develop higher titers to FMDV capsid proteins than to NSPs.[32] Exposure to NSPs occurs when infected cells are lysed,[1] and titers to these proteins seem to be correlated with the extent of virus replication.[33] These antibodies may be transient and difficult to detect in some vaccinated or nonvaccinated animals with low levels of virus replication.[1,34-36] Studies in animals immunized with conventional inactivated vaccines suggest that, for this reason, the current NSP tests should be used as herd level tests, and are not sensitive or specific enough for use as single-stand alone tests in individual animals.[1,32,34,37]

After vaccination, immune responses develop only to the FMDV proteins incorporated into the vaccine. The absence of detectable responses to certain NSPs (e.g., 3B) can be used in serological tests that differentiate vaccinated from infected animals (DIVA tests). The duration of immune responses to different NSPs seems to vary; however, detectable antibody titers to 3ABC were reported to persist for 1-2 years.[1,32,34] The low antibody titers to NSPs in vaccinated animals should be considered when evaluating the use of serological DIVA tests with any vaccine. These assays need to be validated for each system and host species.

2.3 Carriers

Some ruminants become carriers after recovery. An FMDV "carrier" is defined as an animal with persistent virus or viral genome in the pharyngeal region for longer than 28 days.[38] The epidemiological significance of FMDV carriers among cattle, sheep and goats is controversial, as it is uncertain whether they can transmit the virus, and if so, under what circumstances.[17,39-42] Unequivocal evidence for transmission from carriers has been reported only for the SAT viruses in African buffalo (*Syncerus caffer*),[39,40,43] and the Royal Society, London concluded in 2002 that the risk of transmission from domesticated animal carriers appears to be very low, if it occurs at all.[42 cited in 44] The current consensus is that pigs do not become carriers.[17,34,39,40]

2.4 Duration of immunity

There is only limited information on how long immunity persists after an animal recovers from FMD. Some factors that may affect the duration of immunity (DOI) after

FMDV infection include the host species, individual animal variability and the virulence of the virus strain.[18] Some studies suggests that immunity can last for at least 6 months in cattle, and possibly longer in some individuals. In one experiment, cattle were protected from clinical signs when they were challenged with the homologous virus 6 months after infection.[18] In a similar study, cattle were protected from disseminated disease one year after infection, although lesions occurred at the inoculation site.[45 cited in 18] A few reports suggest the possibility of longer term protection. In one study, 8 cattle that still had antibody titers to FMDV, 5.5 years after they were infected, did not develop clinical signs when they were challenged with homologous virus.[46 cited in 18] One of 3 cattle at another laboratory was protected from challenge 4.5 years after infection.[45 cited in 18] Antibody levels may also indicate that an animal is immune, although long-term correlation with protection has not been established.[18] Antibody titers have been reported to persist in some vaccinated or infected cattle for up to 5-7 years in some reports.[46-48 cited in 17] A recent study in cattle suggests a possible mechanism for long-term immunity. In this experiment, the FMDV genome and capsid proteins were detected in the germinal centers of lymphoid tissues for up to 38 days after infection; however, the absence of NSPs suggested that these viruses were in a non-replicating state, perhaps in the form of immune complexes or viral particles on follicular dendritic cells.[11]

Immunity to FMDV does not appear to last as long in pigs, possibly because persistent infections do not occur in this species.[18] In pigs, neutralizing antibody titers were reported to peak around 7-10 days after infection, decrease 12-fold, then stabilize around 4 weeks and remain at a plateau for at least 4 months (128 days).[49 cited in 18] Only one of the 5 pigs in this experiment became ill when challenged at 4 months.[18] Other studies in swine reported that approximately half of the animals developed clinical signs when they were re-challenged 3-6 months after infection.[50,51 cited in 18]

Little is known about immunity to FMDV in sheep and goats, but virus neutralizing antibodies first appear 60 hours after virus inoculation in sheep, peak around 10 days, and typically remain at a plateau for at least 147 days (approximately 5 months).[52 cited in 18]

3 CONVENTIONAL INACTIVATED VACCINES

Conventional inactivated FMD vaccines have a long history of use in animals. Modern versions of these vaccines are made from viruses grown in cell culture and inactivated with aziridines.[1,2,17,23] Both aluminum hydroxide and oil adjuvanted vaccines are produced. Aluminum hydroxide/ saponin adjuvanted FMD vaccines are effective in

cattle, sheep and goats, but function poorly in pigs, while oil-adjuvanted (water/oil/water emulsion) vaccines can be used in any species.[2,17,18,33,53,54] Conventional inactivated FMD vaccines can be formulated to produce more potent vaccines (which usually have higher antigen levels) for use in emergency vaccination programs, or less potent vaccines for routine use in endemic areas.[1,23] The viral strain or serotype can influence how much antigen is needed for an effective vaccine. For example, serotype O viruses are less immunogenic than other serotypes, and inactivated vaccines that contain this serotype require a higher antigen payload.[2,18,55,56] Highly purified FMD vaccines, processed to remove NSPs and cell culture proteins, are available from some manufacturers .[1,17,23] However, not all vaccines are purified. Inactivated vaccine antigens can also be concentrated for storage on the vapor phase of liquid nitrogen, and formulated as needed into vaccines.

The viral strains used in inactivated FMD vaccines have traditionally come from field viruses that are adapted to grow in cell culture systems used in manufacturing.[1,2,23] However, some field strains do not grow well in culture.[2] In addition, the adaptation process is time-consuming and expensive, and has the potential to result in antigenic changes during adaptation and *in vitro* growth.[2,17,57,58] The development of vaccine strains by reverse genetics[58-62] might mitigate some of these issues. In one recent study, a vaccine was developed for a serotype A virus that does not grow well in culture, by substituting its P1 genetic sequence into the cDNA clone of a serotype O vaccine strain.[61] In a similar experiment, partial replacements of genetic material were made between field and vaccine strains of SAT viruses.[62] Another group reported making genetic modifications to an infectious cDNA clone of a serotype O vaccine strain, to provide broader protection against three related field viruses.[58] The use of leaderless, inactivated FMDV vaccine constructs, currently in development (see section 4), might also avoid some of the issues with adapting field strains to grow in culture.

Inactivated FMD vaccines are thought to protect animals by inducing humoral immunity, although there is some evidence that they may also stimulate CMI under some conditions,[18,25,63,64] possibly as the result of cross-priming.[65] Inactivated FMD vaccines are not thought to result in mucosal protective immunity,[14,18,66] with the possible exception of certain highly potent vaccines, given repeatedly.[46,67 cited in 17,18] Although vaccinated animals that are protected from clinical signs may transmit FMDV,[68-78] some inactivated FMD vaccines were shown to significantly reduce virus shedding, and decrease or prevent virus transmission in small groups of animals.[58,61,66,68-72,74-85]

3.1 Desirable vaccine characteristics

3.1.1 Rapid onset of immunity

3.1.1.1 Cattle – intramuscular or subcutaneous administration

In experimental challenge systems, inactivated FMD vaccines can sometimes protect cattle from clinical signs as soon as 4-5 days after immunization.[55,68,79] One study suggested that these vaccines may also decrease virus shedding at this time.[82] Other groups reported decreased virus shedding and transmission when animals were challenged at 14 days.[68,75,83] With a severe challenge, Cox et al. (2005, 2007) found that cattle were partially protected from clinical signs 10 days after vaccination, and there was a limited decrease in virus shedding at this time, with improved protection at 3 weeks.[71,72] One study that described early protection (e.g., 5 days) with high potency vaccines reported that both oil and aluminum hydroxide/saponin adjuvants were effective.[55]

Antibodies are also reported to develop relatively soon after vaccination in cattle. Peak antibody titers generally occur 14-28 days after one dose of a conventional inactivated vaccine.[17] In one study, IgM antibodies to FMDV were detected 2-4 days after immunization with an aluminum hydroxide adjuvanted vaccine, and IgG was first reported after 4 days.[86 cited in 18]

3.1.1.2 Cattle – intradermal administration

There is limited information on the onset of immunity following intradermal vaccination, which is under investigation as a means to reduce the antigen dose (section 3.3.1). In one study, 7 cattle received ¼ dose of an inactivated vaccine, administered intradermally with a needle-free device, and were protected from clinical signs, fever and viremia when they were challenged after 7 days.[13] Six of 7 animals that received 1/16 dose of the vaccine, and 5 of 7 animals that received a full dose were also completely protected. In a second trial using the same vaccine, 6 cattle vaccinated with ¼ dose of antigen were completely protected from clinical signs, viremia and fever after challenge at either 7 or 31 days, while cattle vaccinated with 1/16 dose were partially protected at 7 days and completely protected at 31 days.[13] All vaccinated cattle had detectable neutralizing antibody titers by 7 days, with significant titers developing in some animals by this time. These titers increased after challenge, suggesting that the animals were protected from disease, but not from infection.[13]

3.1.1.3 Sheep

In sheep, some vaccines may decrease the shedding and transmission of FMDV as soon as 3-7 days after immunization.[36,67,70,78] Challenge studies conducted 2 or 3 weeks after vaccination also reported decreased virus shedding and transmission.[74,76,84] Protection from illness is more difficult to measure in sheep than cattle or pigs, as even nonvaccinated animals may have few or no clinical signs. However, some clinical protection has been seen as early as 3 or 4 days after vaccination.[36,70]

Neutralizing antibodies have been detected within 7 days in sheep vaccinated with a variety of high potency, oil or aluminum hydroxide adjuvanted, emergency vaccines.[87]

3.1.1.4 Pigs

It appears to be more difficult to protect pigs than ruminants, when they are exposed to FMDV soon after vaccination. Some studies reported that pigs were partially or completely protected from clinical signs as early as 3-4 days after immunization,[69,79,81] and decreased virus shedding and transmission have been observed as soon as 4-7 days.[66,69,81] With a more severe challenge, however, some animals may not be protected even after 2 weeks. Doel et al. (1994) found that only a few pigs were protected from clinical signs if they were challenged 4-16 days after vaccination, but all pigs were protected if challenge occurred after 3-4 weeks.[55] Parida et al. (2007b) reported that most pigs challenged after 10 days became ill, although the clinical signs were less severe than in the controls.[77] If the challenge was delayed until 29 days, 25% of the vaccinated pigs still had mild clinical signs. In this study, the ability of the vaccine to protect pigs from clinical signs was correlated with its ability to decrease virus shedding. Orsel et al. (2007a) also found that some pigs developed clinical signs when they received a severe challenge 2 weeks after vaccination.[88] In this study, vaccination was unable to significantly reduce virus shedding or prevent transmission, although it did decrease the rate of virus transmission. Eble et al. found that a high dose of one vaccine had some effect on virus shedding and transmission in pigs challenged after 7 days, but a lower dose was not protective until 14 days.[66,89]

Neutralizing antibodies were detected within 7 days in pigs vaccinated with high potency, oil adjuvanted, emergency vaccines, and peak antibody titers occurred in 21-28 days.[18,87]

3.1.1.5 Effect of vaccine potency

More potent FMD vaccines are thought to induce immunity more rapidly.[23,54,90] Higher antigen levels usually indicate that an inactivated FMD vaccine is more potent,[54] but the amount of antigen needed to reach a specific level of potency varies with the strain.[28] Limited evidence suggests that, above a certain threshold, increases in antigen concentration might provide little improvement.[73,91] Two issues with increasing the amount of antigen are that fewer vaccine doses are available from a given amount of antigen, and the vaccine is more expensive. This is a particular concern when an emergency vaccine is formulated from limited antigen supplies in a vaccine bank. Boosting less potent vaccines can also be used to increase vaccine efficacy, but immunity develops more slowly than if a single dose of a highly potent vaccine is used.[18,23,90]

The World Organization for Animal Health (OIE) recommends that prophylactic inactivated vaccines, which are used routinely to control FMD in endemic areas, have a minimum potency of 3 PD_{50}[1] per cattle dose.[23] In FMD-endemic areas, vaccinated animals usually have time to develop and maintain an adequate immune response before they are exposed to the virus.[33] They typically receive a booster several weeks after the initial dose, followed by periodic revaccination.[17,23,33,87] Because emergency vaccination may be followed very shortly by challenge,[33] the vaccines used in these campaigns usually contain higher antigen doses to induce a more rapid response. The OIE recommends a PD_{50} greater than 6.[23] Both prophylactic and emergency vaccines supplied by reputable manufacturers and banks usually have PD_{50} levels well over the minimum stipulated values.[33,53,54]

3.1.2 Long duration of immunity

Challenge studies provide the most definitive evidence to support a vaccine's duration of immunity (DOI), but few studies have been published in any livestock species. The maintenance of titers to FMDV for prolonged periods also suggests the persistence of immunity, although it is not conclusive. The DOI for a vaccine may be different in the field than in experiments conducted with healthy young animals under controlled laboratory conditions.

3.1.2.1 Emergency (high potency) FMD vaccines

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[1] For inactivated vaccines, potency is traditionally expressed as the number of 50 percent cattle protective doses (PD50) within each dose of vaccine recommended on the label.

Only a few published studies have evaluated the DOI for emergency (high potency) FMD vaccines, but they suggest that some vaccines may protect cattle, sheep or pigs for at least 6-7 months.[33,73,87,92-94] Cattle were protected from clinical signs, and virus shedding was decreased, when they were challenged 6 months after vaccination with a single dose of a high potency, oil adjuvanted, serotype A vaccine.[73] All animals maintained high antibody titers to FMDV throughout this study, although the titers declined slightly by the day of challenge. Cattle vaccinated with oil adjuvanted SAT vaccines also maintained high titers for at least 6 months.[93] reviewed in [33] However, another study reported that titers in cattle immunized with an oil adjuvanted serotype A vaccine were decreasing by 43 days after vaccination.[94] reviewed in [33]

Pigs immunized with oil adjuvanted, emergency FMD vaccines and challenged after 7 months were protected from clinical signs.[87] Some pigs maintained high antibody titers to FMDV for up to 7 months, although titers in other individuals declined sooner. Another study found that pigs had high antibody titers for at least 6 months after a single dose of vaccine.[92]

Sheep vaccinated with high potency, emergency FMD vaccines maintained titers for up to 6 months,[33,87,92] but no challenge studies have been published. No studies have been published for goats.

3.1.2.2 Prophylactic FMD vaccines

Several field and laboratory studies have evaluated the DOI for prophylactic FMD vaccines. Generally, these vaccines are expected to provide only 4–6 months of immunity,[17,33,95] and animals are re-vaccinated 1-3 times a year, depending on the quality of the vaccine, the epidemiological situation, and the animals' species, life expectancy and economic value.[17,23,87] However, some evidence suggests that, in cattle, the DOI might be prolonged after several doses have been given.[95] In one study, cattle immunized three times with an oil adjuvanted vaccine, at 6 month intervals, did not develop clinical signs when they were challenged 13 months after the last dose.[96] cited in [18] Field studies conducted during routine vaccination campaigns in the Netherlands, in the 1960s, suggested that antibody titers might be maintained for several years.[47] cited in [17,18] During these campaigns, calves lost their antibody titers to FMDV within a few months of the initial vaccination; however, elevated titers were maintained for 12 months after annual revaccination. Lower titers then persisted for 44 months, with little influence of the number of previous vaccinations on the duration of immunity.

Significant antibody titers were also found among vaccinated cattle in France, 6 years after immunization ended.[48 cited in 18]

There are no reports of prolonged DOI in pigs; however, two doses of a prophylactic vaccine, given a month apart, were estimated to provide protection for approximately 6 months, based on serology.[17] A group of goats vaccinated with prophylactic quadrivalent FMD vaccines maintained mean protective titers to serotype O for up to 9 months with an oil adjuvanted formulation, and for up to 6 months with an aluminum hydroxide adjuvanted vaccine.[97]

Some studies[97] and authors suggest that the DOI is longer for oil adjuvanted than aqueous vaccines.[18] Others feel that the DOI for the two adjuvants might be similar,[18] based on certain laboratory studies and the prolonged serological responses in cattle immunized with aluminum hydroxide adjuvanted vaccines in the Netherlands[47] and France.[48]

3.1.3 Absence of interference with booster doses and other vaccines

Conventional inactivated vaccines are given repeatedly in endemic areas, and interference with subsequent FMD vaccinations is not known to occur. Experimental studies[98 cited in 17] and routine batch testing by one company indicate that there is no interference between serotypes or strains when a vaccine contains multiple FMDV strains.[17]

FMD vaccines have been administered simultaneously with many other vaccines including rabies, anthrax and porcine parvovirus, with no apparent effect on either vaccine,[99-102 cited in 17] although this would be difficult to demonstrate conclusively enough to make any regulatory claims.[17]

3.1.4 Effectiveness in the presence of maternal antibody

Animals from non-immune dams can be immunized with inactivated FMD vaccines at 14 days of age, although there is conflicting evidence on whether the response is as effective as in adult cattle.[17]

Maternal antibodies can interfere with inactivated FMD vaccines in calves unless the antibody titer is less than 1:45.[103] The influence of these antibodies can last for several months, and occasionally up to 5-6 months,[104 cited in 17] especially if the dam has been immunized repeatedly.[17] Maternal antibodies were reported to persist for up to 3 months in kids born to goats immunized with commercial quadrivalent FMD vaccines.[97]

In pigs born to vaccinated dams, antibodies can interfere with conventional inactivated FMD vaccines given before 8 weeks of age,[18,105,106] and in some cases, for as long as 10-12 weeks.[107] Maternal antibodies may interfere less with oil adjuvanted vaccines than aluminum hydroxide adjuvanted vaccines in ruminants, although some authors feel the evidence is still inconclusive.[17]

3.1.5 Broad protection within serotypes

Conventional inactivated vaccines provide no protection against other serotypes of FMDV.[18] Cross-reactivity between strains within a serotype varies, and immunodominant vaccine strains can protect animals against some heterologous FMD viruses.[17] Vaccines for serotype A, SAT-1, SAT-2 and SAT-3 viruses, which are antigenically diverse, must contain strains that are closely matched to the outbreak virus.[17,27,28] Some medium and high priority serotype A vaccine strains currently recommended by the WRL FMD for vaccine banking include A_{24} Cruzeiro, A_{22} Iraq, serotype A isolates from Iran (1996, 1999 and 2005), serotype A isolates from Malaysia (1997), Argentina (2001) and Eritrea, and either A Iran 87 or A Saudi 23/86.[17,27] While only one SAT 1 virus and two SAT 2 viruses are currently listed as medium or high priority, this reflects factors other than their genetic diversity (e.g., the availability of vaccine strains within the portfolios of manufacturers able to fulfill the quality requirements for use in Europe).[27] In contrast, coverage for the two major groups of serotype O viruses can be provided by a limited number of vaccine strains.[17,27,28] Important vaccine strains in one of these groups include O Campos, O Lausanne, O BFS 1860 (UK 1967) and O Kaufbeuren,[17] of which either O Campos or O BFS is currently recommended.[27] O Manisa (Turkey 1969) has been the best known vaccine strain in the other group;[17] however, it does not appear to be as protective against some newer viruses, and the new O PanAsia 2 vaccine strain has also been added to the list of vaccines recommended by the WRL FMD.[27] In addition, one vaccine against pig-adapted serotype O Cathay type viruses (e.g., O Taiwan 97) is recommended. One vaccine strain each has been recommended for banking of Asia 1 and serotype C viruses, which are limited in their diversity.[27,28]

Higher potency inactivated vaccines are thought to provide better protection against heterologous strains of FMDV.[23,28,54,90] Initial studies reported at a meeting for representatives of vaccine banks suggest that this effect occurs with some but not all strains.[28] Boosters can also be used to improve the breadth of antigenic cover.[18,23,90] This effect appears to result from increasing the amount of cross-reactive antibodies, rather than by "broadening" the antibody response to other strains.[18] Immunity is not expected to last as long as when the vaccine is well-matched.[18] A recent field study from an outbreak in Israel is illustrative.[108] In this outbreak, a high potency, trivalent vaccine

provided only weak protection against a poorly matched field strain after a few months (with an estimated half-life of protection of 98 days), even in animals that had received multiple boosters; however, a single dose of the same vaccine was protective if the interval between vaccination and exposure was short.[108]

Vaccines generated by reverse genetics may hold promise for inducing broader immunity to some viruses. In one study, genetic modifications made to a serotype O vaccine strain improved protection against 3 related field viruses.[58]

3.1.6 Demonstration of safety and efficacy in multiple species (cattle, swine, sheep, goats)

Conventional inactivated FMD vaccines have been used to vaccinate various species of livestock including cattle, sheep, goats, pigs and water buffalo. In some cases, susceptible animals in zoos have also been immunized regularly with these vaccines.[109] Although many exotic species are expected to respond with antibody titers, there is little experimental information on the ability of FMD vaccines to protect these animals from challenge.[17,109]

The formulation of the vaccine can affect its efficacy and suitability for different species. Aluminum hydroxide/ saponin adjuvanted FMD vaccines are effective in ruminants, but function poorly in pigs, while oil-adjuvanted vaccines can be used in any species.[2,17,33,53,54] Some sources state that FMD vaccines with oil adjuvants are superior to vaccines that have aluminum hydroxide/saponin adjuvants, even in ruminants; however, not all authors or vaccine manufacturers agree.[17] Different formulations of oil emulsions may affect vaccine efficacy.[17]

3.1.7 Knowledge of the level of herd immunity needed to stop transmission in a population (Reproduction ratio)

In cattle, it is believed that at least 80% of the animals must be vaccinated for transmission of FMDV to be prevented in the herd.[17] However, the level of herd immunity varies with its size and the density of the susceptible population, as well as the species within the herd.[110] Thus, the OIE Terrestrial Animal Health Code does not prescribe a specific level of vaccination, although it suggests that immunity in at least 80% of the herd members should be the goal.[110] The reproduction ratio (R) is the average number of secondary infections caused by one infectious individual if the population is completely susceptible. If vaccination decreases R to less than one, the epidemic will die out and only minor outbreaks are expected (however, some transmission is still expected to occur until the epidemic ends). If R remains higher than

1, there can be major outbreaks and the epidemic may continue to grow. Reproduction ratios can be estimated within herds (R0) and between herds (Rh). To date, transmission studies using conventional inactivated FMD vaccines have evaluated R0 but not Rh. However, if vaccination can reduce R0 to less than 1 within a group of animals, "between group" transmission is unlikely.[111 cited in 112]

3.1.7.1 Cattle

In some cases, immunization with a potent inactivated vaccine may decrease R to less than 1 in cattle.[75,83] A single dose of one oil adjuvanted, serotype O vaccine appeared to be capable of halting virus transmission in lactating dairy cows challenged after 2 weeks.[83] R was significantly reduced, from ∞ in nonvaccinated cattle to 0 in vaccinated animals, with no virological or serological evidence that the vaccinated cows shed the virus. In calves, vaccination resulted in a statistically significant decrease in R, from 2.52 in nonvaccinated calves to 0.18.[75] The latter value was significantly less than 1. In another study, cattle vaccinated 3 weeks before challenge did not transmit FMDV to susceptible cattle, while challenge at earlier time points reduced but did not eliminate transmission.[68] Other studies have reported that vaccination decreased virus shedding, but did not evaluate R in transmission studies.[55,71,72,80,82,113-115]

3.1.7.2 Sheep and goats

In one study, R was estimated to be 1.14 in nonvaccinated lambs and 0.22 in vaccinated lambs challenged 2 weeks after immunization.[76] These two values were not significantly different at $P < 0.05$. However, some vaccinated lambs did not become infected after inoculation with FMDV, and transmission could not be evaluated from these animals. This, together with the low R value in the nonvaccinated group, may have accounted for the failure to reach statistical significance. In another study, where the interval between vaccination and challenge was 3-10 days, transmission between sheep was reduced or prevented by either an oil or an aqueous C_1 Oberbayern vaccine, but animals immunized with an oil adjuvanted Asia 1 vaccine transmitted the homologous virus to susceptible contacts.[70] Other studies reported decreased virus shedding in vaccinated sheep or goats, but did not evaluate transmission.[36,74,78,84]

3.1.7.3 Pigs

Some studies in pigs have reported that vaccination can decrease, though not always eliminate, virus transmission to contacts.[66,116-120] Eble et al. (2004, 2006) found that,

when pigs were vaccinated 2 weeks before challenge, susceptible contacts did not become infected, and R was significantly lower than in nonvaccinated pigs.[66,117] A meta-analysis of several experiments from this group suggested that, if pigs were challenged 7 days after immunization, R was significantly reduced in pigs vaccinated with a four-fold-dose of vaccine, but not in pigs vaccinated with a single dose.[118] Other studies reported that vaccination prevented transmission in pigs challenged at least 7 days later, but not at earlier time points.[69,81] One study found that R remained above 1 if pigs received a severe challenge 2 weeks after vaccination, although the transmission rate was reduced.[88] In this study, R was ∞ in nonvaccinated pigs and 2.42 in vaccinated pigs , but the difference was not statistically significant. However, the transmission rate (β) was significantly lower in vaccinated pigs (6.84/ day) than nonvaccinated pigs (0.66/ day), suggesting that immunization might slow virus spread.

Two field studies in pigs suggest that vaccination might be able to suppress virus transmission sufficiently to eradicate it in isolated swine herds. Poulin and Christianson (2006) found that FMD could be controlled in a closed pig herd by vaccination and strict biosecurity.[119] Eradication was achieved after one year, and the virus did not spread to other herds. Chen at al. (2008) reported similar results in one closed pig herd infected with O/Taiwan/97.[120]

3.2 Desirable characteristics for vaccine manufacture and stockpiling

3.2.1 Safety for manufacturing under BL2 conditions in the U.S.

Conventional inactivated vaccines cannot be manufactured under BL2 conditions in an FMD-free country, as these vaccines are manufactured by growing live, virulent FMDV in culture.[1,2,23] Secure facilities, with strict containment precautions to prevent virus release, are required for vaccine production.[17] Although reports of accidental release have become rare with modern biosecurity standards,[18] the possibility cannot be entirely eliminated. In 2007, a limited outbreak in the U.K. was apparently caused by a vaccine virus that escaped from a laboratory.[121]

3.2.2 Vaccine organism is not a select agent

FMDV is considered to be a "Tier 1 select agent" by the U.S. government,[122] and strict rules govern research with this organism. In addition, it is illegal to possess live FMDV on the U.S. mainland, and it is thus currently impossible to manufacture conventional inactivated vaccines in the U.S.[123]

3.2.3 Capability of meeting 9 CFR regulatory requirements for purity, potency, safety, and efficacy

The Department of Homeland Security (DHS) has provided funding to enable one FMD vaccine produced outside the U.S. (a quadrivalent FMD vaccine produced by Biogenesis Bago in Argentina) to be permitted for distribution and sale as part of an official USDA animal disease control program. Identification of other overseas qualified, FMD vaccine manufacturers interested in this licensing pathway are in progress (personal communication from personnel at DHS).

3.2.4 Capability of meeting requirements for cost effective manufacturing and all proprietary rights to vaccine antigen, vectors, and/or adjuvants

A number of conventional inactivated FMD vaccines are produced routinely by qualified companies outside the U.S. These vaccines are clearly capable of meeting both requirements.

3.2.5 Desirable characteristics for stockpiling for emergency use

3.2.5.1 Ongoing manufacture and sale in endemic countries which enables indefinite delivery/indefinite quantity (IDIQ) contracts for just in time delivery

DHS has provided funding to enable one FMD vaccine to be permitted for distribution and sale in the U.S., under the supervision and control of USDA, APHIS, Veterinary Services, and as part of an official USDA animal disease control program. The vaccine is a quadrivalent FMD vaccine (for serotypes A_{24} Cruzeiro, A2001 Argentina, C_3 Indaial, and O_1 Campos) produced by Biogenesis Bago in Argentina. The manufacturer produces sufficient vaccine to meet its current customers' needs (personal analysis, J.A. Roth, based on information from vaccine company personnel). It does not maintain stocks of this vaccine that could be immediately available in sufficient quantity for rapid use in controlling even a small outbreak. The manufacturer would need to increase production once a need became apparent. Several weeks would be required to begin to produce vaccine, and several months (or years) to produce sufficient vaccine to meet the potential need. Alternatively, an indefinite delivery/ indefinite quantity contract could be negotiated with the manufacturer to ensure that a specific number of doses was always available for emergency use in the U.S.

3.2.5.2 Stability when stored as bulk antigen

Concentrated, purified FMDV antigens can be stored for prolonged periods before formulation into complete vaccines. These antigens can be frozen at ultra-low temperatures (-70°C or lower) in vaccine banks for at least 5 years,[9] and in some cases, for more than 15 years.[124] Strain- or serotype- related differences may affect vaccine manufacture and storage. For example, SAT-1, SAT-2, and SAT-3 viruses are less stable than other serotypes,[2] and extra quality assurance steps must be taken to ensure that vaccines containing these serotypes are potent and remain so during storage.[2]

3.2.5.3 Long shelf-life and stability of finished vaccine

Once formulated, the shelf life of conventional inactivated FMD vaccines is usually 1-2 years at a temperature of 2-8°C.[23] Some emergency vaccines may be less stable than prophylactic vaccines.[54] This effect, which has been reported for some FMD vaccines but not others, might be caused by proteases from the culture harvest and/or the type of formulation.[54]

Conventional inactivated FMD vaccines (final formulation) are temperature labile, and current guidelines indicate that they should not be frozen or stored above the target temperature.[23]

3.2.5.4 Rapid scale up and manufacture in the US in an emergency
Because it is currently illegal to possess FMDV on the mainland, conventional inactivated vaccines cannot be manufactured in the U.S. (see section 3.2.1).

3.2.6 Rapid conversion of manufacturing to the outbreak strain
An appropriate vaccine strain can be identified quickly during an outbreak, if one is available.[17]. For example, this process took only a few days during the 2001 FMD outbreak in the U.K.[17] Although novel strains of some serotypes emerge and disappear regularly,[29] outbreak strains that are not covered at all by existing stocks of inactivated vaccines (e.g., the A_{22} strain that emerged in the 1950s) appear very rarely.[28] Continuous monitoring of FMDV field isolates and periodic development of new vaccine strains can mitigate issues with the diversity of FMDV strains.[17] One European manufacturer maintains approximately 25 master seed viruses (MSVs) in its laboratory.[17]

New vaccine strains may, nonetheless, need to be produced for an outbreak or routine use, either because no reasonably well-matched vaccine strain is available, or to optimize vaccine efficacy. MSVs have traditionally been made by adapting field viruses

to culture, via passage in a suitable cell line.[17] The number of passages necessary to produce a high yielding, efficacious MSV varies with the strain.[17] Some FMD viruses may be difficult to adapt to culture (e.g., they fail to adapt to suspension culture, grow slowly, have low yields or have a tendency to aggregate), or the process may result in antigenic changes.[2,28] In addition, the quality or number of field strains from an outbreak might be inadequate.[28] If the adaptation of a field strain is successful, the lead time for vaccine preparation is estimated to be 1 to 8 months, depending on how readily the strain grows *in vitro*, its yield and immunogenicity, and the licensing tests that must be conducted.[28,53 and personal communication, personnel at USDA APHIS] A possible alternative approach, currently under investigation, is to develop new vaccine strains by modifying cDNA clones of existing strains.[58-62]

If a new strain must be developed, the most closely related vaccine strain, or combination of related strains, might be used for control programs in the interim.[28 and personal communication, personnel at USDA APHIS] Increasing the concentration of the antigen, as well as double sequential vaccination, could be used to increase a heterologous vaccine's potency.[28] However, repeated vaccination cannot overcome large antigenic differences; in this case, only a new vaccine strain is expected to provide reasonable efficacy.[17]

3.3 Desirable characteristics for vaccine administration

3.3.1 Cost effective and practical delivery methods
Effective vaccination programs require adequate supplies of vaccination equipment. For conventional inactivated vaccines, this includes sterile needles and syringes, and cool boxes to keep the vaccine at a temperature of 3-8°C, in addition to other equipment needed in all vaccination campaigns (e.g., restraints to allow safe application, ear tags, protective clothing and disinfectants).[17] Aqueous FMD vaccines are usually administered by subcutaneous inoculation, while vaccines with oil adjuvants are usually given to both ruminants and pigs by the intramuscular route.[17]

Intradermal inoculation methods in development may permit the antigen dose per animal to be reduced, thus increasing the number of animals that can be vaccinated from limited antigen supplies. Vaccines administered by this route seem to be more immunogenic, probably due to the large numbers of dendritic cells in the skin.[125;126,127 cited in 13] Another advantage to intradermal needle-free devices is that they can accommodate multiple doses, increasing the efficiency of vaccine administration compared to needle and syringe.[13] In early experiments, such devices appear to be promising. In one study, 1/4 dose of an aqueous FMD vaccine was as effective as a full dose, when both were administered intradermally with a needle-free, compressed gas

vaccination system (Dermavac®) to cattle.[13] All 7 cattle vaccinated with ¼ dose, and 5 of 7 animals vaccinated with a full dose, were protected from clinical signs if they were challenged in 7 days. A further reduction to 1/16 dose seemed to be less effective when challenge occurred 7 days after vaccination; however, this dose protected all animals if the challenge was delayed until 31 days.[13] Intradermal, needle-free vaccination of pigs with 1/10 dose of an oil adjuvanted FMD vaccine was also promising; however, optimization of the dose could not be achieved.[128] In this experiment, protection was similar whether pigs were vaccinated intramuscularly with a full vaccine dose or intradermally with a 1/10 dose. The viscosity of oil adjuvants could be a concern with these devices.

3.3.2 Safety for humans accidentally exposed to the vaccine

There is no evidence that the antigens in inactivated FMD viruses are a safety hazard for humans.[129] Local reactions from oil adjuvants or other ingredients are addressed in label warnings.[130]

3.4 Desirable characteristics for minimizing the impact on food production and animal trade

3.4.1 Safety for use in food producing animals with no, or reasonably short, withdrawal time for animal products for human consumption

3.4.1.1 Absence of virulent virus and other animal pathogens

Properly inactivated FMD vaccines produced under good quality control conditions are expected to be safe for use in food producing animals. Some vaccine-related outbreaks occurred in the past when formaldehyde, which has an exponential inactivation curve, was employed for virus inactivation.[1] Modern vaccine facilities typically use aziridines (binary ethyleneimine), which inactivate FMDV more effectively.[1,2,17,23] Time and temperature conditions must be validated for the conditions and equipment, and the rate of inactivation is determined for every batch of antigen.[23] With the current system, it is possible to achieve the Ph.Eur standards of less than 1 infectious particle per 10,000 liters of FMD antigen preparation.[17] One European manufacturer states that residual infectivity has never been detected during *in vitro* tests after inactivation.[17] After purification, this manufacturer evaluates the concentrated antigens in a second innocuity test, using at least 200 cattle dose equivalents to inoculate FMDV-susceptible cell monolayers, followed by two sequential passages of the cells.[17]

During manufacture, MSVs and master cell stocks (MCSs) are tested for freedom from contaminating microorganisms including mycoplasma, bacteria, fungi and viruses.[17] Starting materials of biological origin are usually tested for the absence of adventitious agents or obtained as gamma irradiated products.[17] The vaccine is also tested for identity, to ensure that only the selected strain is present.[17] Lastly, the final product safety test in animals aids in ensuring innocuity.[17]

3.4.1.2 Allergic reactions in vaccinated animals

A number of reports described allergic reactions (some serious or fatal) in animals immunized with FMD vaccines during the 1970s.[131 cited in 17] Potential causes included the use of formaldehyde (which may modify extraneous proteins in crude antigen harvests), the quality of the saponin and the amount of protein in the vaccine.[17] In particular, some polyvalent vaccines may initially contain high concentrations of extraneous proteins from the cell culture, increasing the risk of adverse reactions unless the FMD antigens are purified.[17] Hypersensitivity reactions are reported to be unlikely with vaccines that contain purified components and are inactivated with binary ethyleneimine,[17] although such reactions (including severe reactions) are still reported occasionally.[132]

3.4.1.3 Withdrawal period and safety for human consumption

All vaccines for food animals in the U.S. must be labeled with a minimum slaughter withdrawal time of 21 days. Oil-adjuvanted vaccines cause local injection site inflammation and usually have a 60-day slaughter withholding time.

The U.K. Food Standards Agency has stated that there is no risk to human health from eating products from animals that have been vaccinated with an approved FMD vaccine, and that there is no need to label such products separately.[129]

3.4.2 Availability of companion diagnostic test to detect infections in vaccinated animals (DIVA)

Inactivated FMD vaccines primarily induce antibodies to the viral structural proteins.[1] Unpurified vaccines also contain low levels of NSPs, and may result in titers to these proteins, especially when animals are vaccinated repeatedly.[34] If, however, an inactivated vaccine is sufficiently purified, vaccinated animals should be exposed to most NSPs [1,9] only if they become infected with a field virus. (The 3D protein, which is

incorporated into the capsid, is an exception.) Some manufacturers state that they concentrate and purify conventional inactivated vaccines to a high degree, and significant antibody titers to NSPs do not develop after repeated vaccination.[17]

ELISAs and the enzyme-linked immuno-electrotransfer blot (EITB) can be used as DIVA (NSP) tests with conventional inactivated vaccines.[23] Most ELISAs are based on the 3AB or 3ABC proteins, as studies in experimentally infected cattle have found that this NSP induces the most reliable serological reactions.[133,134] Because vaccination often reduces virus replication, antibody titers to NSPs tend to be lower in vaccinated than nonvaccinated animals, and seroconversion can be delayed or even absent.[1,32,36,123,135,136] For this reason, current NSP tests are generally considered to be valid only as herd level tests, and are not sensitive or specific enough for use as, single-stand alone tests in individual animals.[1,32,34,37] NSP DIVA tests can be used with conventional inactivated vaccines as part of the procedure to regain OIE FMD-free status.[1,32,34,37,110]

3.5 Desirable characteristics for controlling FMD in wild and feral populations

3.5.1 Safety and efficacy when delivered orally in baits for feral swine or deer

Oral vaccines for feral and wild animals susceptible to FMDV may be desirable; however, none are currently available and none appear to be in development. Developing oral vaccines for any disease is a challenge, as the environment of the gastrointestinal tract degrades the epitopes in most soluble antigens, mucosal tolerance can be difficult to overcome, antigens may be transported poorly across the intestinal epithelium, and interactions can occur between the antigen and the normal flora or other GI components.[137,138] Nevertheless, it is possible to develop effective vaccines, although currently there is poor understanding of why some oral vaccines work, while other vaccines are ineffective.[137,138] Prolonged exposure to the antigen and high antigen doses are usually required to induce an immune response.[137]

Although some non-living vaccines have been used successfully in mucosal vaccination,[137,138] there are no published reports suggesting that conventional inactivated FMD vaccines would be effective in inducing immunity by the oral route.

4. INACTIVATED LEADERLESS LL3B3D FMD VACCINES

An FMDV construct termed FMD-LL3B3D, which has a deletion in the leader protease (L^{pro}) gene and two marker mutations in the 3B and 3D non-structural genes, is in development as a safer platform for the production of inactivated vaccines.[139] Ideally, the leader deletion results in viruses that can still replicate in culture, but do not cause disease in FMD-susceptible animals. The substitution of these viruses for virulent, cell culture adapted field viruses in the manufacturing process may make it possible to produce inactivated FMD vaccines in the U.S. at a BSL-2 level.

Although the entire L^{pro} coding sequence cannot be deleted without resulting in a non-viable virus,[139,140] a partial deletion has been successfully made in a serotype A_{24} Cruzeiro virus. The leader protease can be translated from two different AUG translation initiation sites, resulting in two different proteins (Lab and Lb).[139,140] The utilization of the two initiation sites differs between strains of FMDV.[140] At least two serotype A constructs that do not produce functional L^{pro}, but retain the inter-AUG sequence and the second initiation codon, are infectious but propagate more slowly in cell culture, and are attenuated in cattle and pigs.[139,141-143] The slower replication is thought to result from some loss of their competitive advantage over cellular mRNA.[141] The FMD-LL3B3D A_{24} Cruzeiro construct has been further modified by introducing unique restriction endonuclease sites on either side of the capsid coding region, which allow these structural gene sequences to be changed readily.[139] DIVA capability has been enhanced by replacing immunodominant epitopes in the NSPs 3B and 3D with sequences corresponding to bovine rhinitis virus 2.[139] These marker mutations allow serological reactions to NSPs in field viruses to be distinguished from reactions to the vaccine strain, even when the vaccine is unpurified.[139] The resulting vaccine construct is designated FMD-LL3B3D A_{24} Cruzeiro. Other constructs that utilize the FMD-LL3B3D backbone are being developed for O_1 Campos, C_3 Indaial, Asia 1 Shamir, and several other FMDV strains (personal communication from personnel at Zoetis, Inc.).

Inactivated vaccines produced from FMD-LL3B3D A_{24} Cruzeiro or its derivatives would share many of the characteristics of conventional inactivated FMD vaccines, but with increased safety during manufacture. FMD-LL3B3D A_{24} Cruzeiro vaccine candidates have been evaluated with a conventional oil adjuvant,[139] as well as with a proprietary adjuvant that contains immunomodulatory factors (personal communication from personnel at Zoetis, Inc.). Vaccines containing both adjuvants protected cattle from clinical signs and viremia, and induced humoral immune responses, but the use of the proprietary adjuvant resulted in higher antibody responses, and was also reported to increase CMI responses compared to a commercial, conventional inactivated FMD vaccine.[139 and personal communication from personnel at Zoetis, Inc.]

4.1 Desirable vaccine characteristics

4.1.1 Rapid onset of immunity

Inactivated FMD-LL3B3D vaccines would probably have an onset of immunity similar to conventional inactivated FMD vaccines using the same adjuvants, but protection from challenge within the first 2 weeks after immunization has not yet been examined. In the single published study, which employed an oil adjuvant, cattle did not develop clinical signs or detectable viremia, when they were challenged 3 weeks after receiving one dose of inactivated FMD-LL3B3D A_{24} Cruzeiro vaccine.[139] However, serology suggests that animals might be protected sooner. Four of the 5 cattle had neutralizing antibodies by day 7, and the remaining animal developed neutralizing antibodies by day 14. In recent, unpublished studies utilizing the FMD-LL3B3D A_{24} Cruzeiro antigen with a proprietary adjuvant, cattle developed significantly higher ($P<0.05$) neutralizing antibodies by day 7, compared to cattle that received the same vaccine with a commercial adjuvant (personal communication from personnel at Zoetis, Inc.). These cattle likely produced protective neutralizing antibody titers within 3-4 days (personal communication from personnel at Zoetis, Inc.).

There is currently no information on the use of FMD-LL3B3D vaccine constructs in swine. In an earlier study, pigs vaccinated with a similar inactivated, oil adjuvanted, leaderless serotype A_{12} construct developed neutralizing antibodies by day 7, and these antibodies peaked 14-21 days after vaccination.[143] The pigs were also protected from clinical signs after challenge, but the challenge did not occur until 8 weeks.

4.1.2 Long duration of immunity

No studies have evaluated the DOI for FMD-LL3B3D vaccine candidates. It would probably be similar to the DOI for other inactivated FMD vaccines with the same adjuvants. It is possible that the use of the proprietary adjuvant would increase the DOI for inactivated FMD-LL3B3D vaccines.

4.1.3 Absence of interference with booster doses and other vaccines

Inactivated vaccines generated from FMD-LL3B3D constructs would be expected to function similarly to conventional inactivated vaccines, which can be administered repeatedly without interfering with FMD boosters or other vaccines.[17]

4.1.4 Effectiveness in the presence of maternal antibody

As with vaccines produced from inactivated field strains of FMDV,[17,18,23,103,105] maternal antibodies would be expected to interfere with vaccination in young animals.

4.1.5 Broad protection within serotypes

The efficacy of FMD-LL3B3D vaccines against heterologous viruses is likely to resemble that of conventional inactivated vaccines with the same adjuvant, if the construct contains unmodified FMDV capsid sequences. Some genetic modifications of the capsid coding region might result in broader immunity, as has been demonstrated for conventional inactivated vaccines made by reverse genetics.[58] The induction of better CMI with a proprietary adjuvant (unpublished experiments, personal communication from personnel at Zoetis, Inc.) might also improve cross-reactivity.

Producing FMD-LL3B3D vaccines as unpurified vaccines might result in stronger and more cross-reactive immune responses from the inclusion of NSPs.[139] Unpurified vaccines could be produced without losing DIVA capability, due to the marker mutations in 3B and 3D.[139] This theoretical advantage should be balanced against other considerations, such as possible increases in adverse reactions (e.g. hypersensitivity reactions) from cell culture proteins in unpurified vaccines (section 4.14.2).

4.1.6 Demonstration of safety and efficacy in multiple species (cattle, swine, sheep, goats)

Inactivated vaccines produced from FMD-LL3B3D vaccine constructs that utilize existing adjuvant systems would not be expected to differ from conventional inactivated vaccines, which have been used in numerous species including both livestock and exotic animals in zoos.[17,109] Vaccines with proprietary adjuvants would need to be tested for safety and efficacy in the various target species, particularly exotic and zoo animals. Currently, these experimental vaccine candidates have been evaluated only in cattle.

4.1.7 Knowledge of the level of herd immunity needed to stop transmission in a population (Reproduction ratio)

The ability of conventional inactivated FMD vaccines to reduce transmission varies with the vaccine, host species, challenge dose and other factors.[36,55,66,68-72,74-78,80-84,88,89,113-115,118] Similarly, the effect of inactivated leaderless vaccines would likely depend on the specific construct; the antigen concentration and other factors involved in vaccine efficacy; and the species of the animals and challenge dose. No transmission studies have been published yet for these vaccines; however, unpublished studies

demonstrated that the FMD-LL3B3D A$_{24}$ Cruzeiro construct significantly reduced nasal shedding after challenge at 3 weeks (personal communication from personnel at Zoetis, Inc.). This was seen with both the oil adjuvant and the proprietary adjuvant.

4.2 Desirable characteristics for vaccine manufacture and stockpiling

4.2.1 Safety for manufacturing under BL2 conditions in the U.S.

Live FMD-LL3B3D A$_{24}$ Cruzeiro vaccine viruses appear to be much safer to grow in culture than vaccine strains made from field viruses; there may be little or no risk to livestock if these viruses are accidentally released before inactivation. Similar serotype A (A$_{12}$) constructs were originally developed for use as live attenuated vaccines in cattle and pigs.[141-143] These vaccines did not cause clinical signs in small numbers of cattle and pigs, and the vaccine viruses were not transmitted to FMDV-naive animals.[141,143,144] The FMD-LL3B3D A$_{24}$ Cruzeiro construct was recently tested for virulence in cattle and swine.[139] One of two pigs inoculated into the heel bulbs developed viremia and shed small amounts of virus in nasal secretions; however, neither pig transmitted the virus to nonvaccinated contacts or developed clinical signs. No viruses were detected in air samples from the room. Three cattle exposed via aerosols and 2 cattle inoculated intradermolingually did not develop viremia or clinical signs, and virus shedding was not seen.[139] Antibody responses were low or absent in all of the animals, suggesting that there was little or no virus replication. Although these initial studies are promising, it will be important to confirm the results in larger numbers of animals, particularly pigs, and during field testing. The FMD-LL3B3D A$_{24}$ Cruzeiro construct should also be evaluated in FMDV-susceptible species other than cattle and pigs (e.g., sheep). It is possible for a leaderless vaccine to be attenuated in one species, but retain some virulence in another.[142]

The innocuity of FMD-LL3B3D vaccine constructs that incorporate capsid coding sequences other than A$_{24}$ Cruzeiro should also be demonstrated. In previous studies with leaderless serotype A$_{12}$ viruses, or these constructs substituted with type O capsid sequences, some constructs were more attenuated than others in pigs (see also section 4.2.6).[142] However, preliminary studies with other FMD-LL3B3D constructs have yielded similar safety profiles to FMD-LL3B3D A$_{24}$ Cruzeiro (personal communication from personnel at Zoetis, Inc.).

The possibility of reversion to virulence must always be considered with attenuated viruses. Viruses that have been attenuated by passage in non-host species or cell cultures may differ from wild-type viruses by only small changes (e.g., point mutations). Further mutations could allow these viruses to recover their virulence. The deletion of

large segments of the FMDV genome in leaderless FMD vaccines makes reversion to virulence much less likely.[141] There has also been no evidence of reversion to virulence in studies with the FMD-LL3B3D A_{24} Cruzeiro construct in cattle (personal communication from personnel at Zoetis, Inc.). In addition, a cattle recombination study (submitted at the request of the Select Agent Program as part of the SAP Exclusion request; see section 4.2.2) demonstrated that co-infection of FMD-LL3B3D A_{24} Cruzeiro and the closest FMDV relative, bovine rhinitis virus, failed to yield any evidence of recombination between the two viruses, or reversion to virulence.

4.2.1.1 Considerations for potency testing during vaccine manufacture

One challenge for any vaccine produced in the U.S. is the need to conduct potency testing. Potency tests for conventional inactivated vaccines (e.g., the PD_{50} or PGP test) are animal challenge tests, and require the use of live, virulent FMDV.[23] Even if the vaccine itself can be manufactured on the U.S. mainland, these tests must be done at secure facilities, most likely at Plum Island Animal Disease Center. Indirect tests, such as the measurement of FMDV-specific antibody titers, can be used to measure potency for batch release if there is a satisfactory correlation between the test results and protection in the target species.[23,145,146]

4.2.2 Vaccine organism is not a select agent

Although FMD viruses are currently classified as select agents,[122] an attempt to remove the FMD-LL3B3D A_{24} Cruzeiro vaccine construct (and eventually other constructs) from this category is underway. It is critical to successful development of this platform that the FMD-LL3B3D-based vaccine strains be excluded from the Select Agent Program regulations.

4.2.3 Capability of meeting 9 CFR regulatory requirements for purity, potency, safety, and efficacy

Manufacture of inactivated vaccines based on the FMD-LL3B3D construct should be similar to other inactivated FMD vaccines, some of which appear to be capable of meeting 9 CFR requirements. At least one inactivated vaccine is being considered for import into the U.S., if needed during an outbreak.

4.2.4 Capability of meeting requirements for cost effective manufacturing and all proprietary rights to vaccine antigen, vectors, and/or adjuvants

The FMD-LL3B3D vaccine construct should be able to meet all requirements for cost effective manufacturing and all proprietary rights to the vaccine antigen, vectors and/or adjuvants. One potential cost advantage is that purification is not necessary for DIVA capability.[57,139] The ability to manufacture FMD vaccines without high biosecurity level facilities may also decrease costs.

4.2.5 Desirable characteristics for stockpiling for emergency use

4.2.5.1 Ongoing manufacture and sale in endemic countries which enables indefinite delivery/ indefinite quantity (IDIQ) contracts for just in time delivery

No FMD-LL3B3D vaccines are currently manufactured or used in any endemic countries. This could be a possibility in the future.

4.2.5.2 Stability when stored as bulk antigen

The FMD capsid proteins are not modified in the FMD-LL3B3D A_{24} Cruzeiro vaccine construct. Their stability should thus be similar to antigens from field viruses.

4.2.5.3 Long shelf-life and stability of finished vaccine

Formulation of an inactivated vaccine from an FMD-LL3B3D vaccine construct would be similar to a conventional inactivated FMDV vaccine, and its properties should be similar, when current adjuvant systems are utilized. This would include temperature lability (requiring maintenance at a target temperature of 4°C) and an estimated shelf-life of 1-2 years.[23] The use of proprietary adjuvant systems or drying technologies may provide increased shelf life (personal communication from personnel at Zoetis, Inc.).

4.2.5.4 Rapid scale up and manufacture in the US in an emergency

It should be possible to produce inactivated vaccines from FMD-LL3B3D constructs in the U.S., provided that rigorous testing confirms the absence of disease and virus transmission from susceptible species inoculated with the live construct (see also section 4.2.1). Vaccine companies would need to be pre-approved to produce the vaccine and would need to have approved MCSs and MSVs ready for use. If no vaccine were stockpiled, one South American FMD vaccine company estimated that several weeks would be required for it to begin to produce a conventional inactivated vaccine

for an outbreak in the U.S., and several months (or years) to produce sufficient vaccine to meet the potential need (personal analysis, J.A. Roth, based on information from vaccine company personnel). A similar or longer timeframe might be applicable for a single U.S. company, which was not previously producing the FMD-LL3B3D inactivated vaccine, to convert production. If matching FMD-LL3B3D vaccines are being produced for overseas use, Zoetis, Inc. anticipates that production of vaccines for use in a U.S. outbreak would be much quicker (personal communication). However, constraints such as contracts with other clients would need to be anticipated.

4.2.6 Rapid conversion of manufacturing to outbreak strain

Unique restriction endonuclease sites flank the capsid coding region in the FMD-LL3B3D vaccine construct, and allow this sequence to be changed readily to that of an outbreak strain.[139] Although the FMD-LL3B3D vaccine construct should theoretically work for other serotypes and strains, there may be some constraints in practice. In other studies, a leaderless serotype A_{12} construct was difficult to adapt for a serotype O virus.[142] When the capsid coding sequence for a virulent serotype O_1 Campos virus was inserted into the A_{12} construct, the resulting live vaccine strain still had some virulence for pigs, and was transmitted between these animals, although it appeared to be avirulent in a steer.[142] A capsid coding sequence for a mutated, tissue culture-adapted, attenuated O_1 Campos virus was avirulent in pigs, but it was less immunogenic and did not protect pigs from challenge.[142] Preliminary results suggest that this might not be a problem with the FMD-LL3B3D platform. An FMD-LL3B3D O_1 Campos construct produced no disease symptoms when it was inoculated into pigs (personal communication from personnel at Zoetis, Inc.). Vaccination studies with inactivated viral antigen are still required to demonstrate immunogenicity of this construct.

Full evaluation of each construct for U.S regulatory approval would be expected to take 3-5 years[2] unless a conditional license is issued. Vaccines with new FMDV antigens might become available sooner, if the FMD-LL3B3D vaccines qualify for inclusion as a production platform under APHIS guidelines for licensing production platforms (Veterinary Services Memorandum 800.213).

4.3 Desirable characteristics for vaccine administration

4.3.1 Cost effective and practical delivery methods

Inactivated FMD vaccines are injected subcutaneously, if using aluminum hydroxide adjuvant, or intramuscularly if the adjuvant is oil.[17] As with other inactivated vaccines, a

reliable cold chain would be necessary.[17,23] Needle-free devices with reduced antigen doses are under investigation for conventional inactivated vaccines[13,128] and might also be used with inactivated vaccines produced from FMD LL3B3D constructs.

4.3.2 Safety for humans accidentally exposed to the vaccine

There is no evidence that the antigens in inactivated FMD viruses are a safety hazard for humans.[129] Local reactions from oil adjuvants or other ingredients should be addressed in label warnings.[130]

4.4. Desirable characteristics for minimizing the impact on food production and animal trade

4.4.1 Safety for use in food producing animals with no, or reasonably short, withdrawal time for animal products for human consumption

4.4.1.1 Absence of virulent virus and other animal pathogens

The same inactivation protocols and quality controls used for other inactivated vaccines would also be effective for ensuring the safety of FMDLL3B3D vaccine constructs. In the remote possibility that any residual viruses remained in an FMD-LL3B3D A_{24} Cruzeiro vaccine, they would be attenuated for cattle and pigs.[139,141-143] The complete deletion of large segments of the genome substantially reduces the risk that the FMD LL3B3D vaccine construct can revert to virulence.[141] Reversion to virulence studies with the FMD-LL3B3D A_{24} Cruzeiro strain have also demonstrated stability of the construct in cattle.

4.4.1.2 Allergic reactions in vaccinated animals

The risk of hypersensitivity reactions would most likely be similar to conventional inactivated FMD vaccines of the same type. In unpurified vaccines, high concentrations of extraneous proteins from cell culture might increase the risk of adverse reactions.[17]

4.4.1.3 Withdrawal period and safety for human consumption

The withdrawal period for an inactivated FMD-LL3B3D A_{24} Cruzeiro vaccine and a conventional inactivated FMD vaccine with the same adjuvant would probably be

identical. Products from animals vaccinated with inactivated FMD vaccines are considered safe for human consumption.[129]

4.4.2 Availability of companion diagnostic test to detect infections in vaccinated animals (DIVA)

The 3B and 3D NSPs in FMD-LL3B3D vaccines contain marker mutations that allow the use of DIVA tests, even without purification of the vaccine antigens.[139] Companion serological DIVA tests (e.g., competitive ELISAs),[57]) would be based on these altered epitopes.[139] Because the negative antigenic markers are built into the platform, multiple boosts with FMD-LL3B3D derived vaccines would not generate antibodies that would interfere with the DIVA-compatible assay. PCR targeted to the deleted leader region could be used to distinguish the vaccine construct from field viruses.[139]

DIVA capability would be expected to allow FMD-LL3B3D vaccines to be used with surveillance programs intended to document freedom from infection for international trade. However, this will require validation of the DIVA assays for this purpose.

4.5 Desirable characteristics for controlling FMD in wild and feral populations

4.5.1 Safety and efficacy when delivered orally in baits for feral swine or deer

Although some non-living vaccines have been used successfully in mucosal vaccination,[137,138] there are no published reports suggesting that inactivated FMD vaccines would be effective in inducing immunity by the oral route.

5. HUMAN ADENOVIRUS 5 VECTORED FMD VACCINES

Adenoviruses (family Adenoviridae) are non-enveloped viruses with double-stranded DNA genomes. Vectors generated from human adenovirus 5 (hAd5), a mild respiratory pathogen of people,[147] have been used extensively in experimental vaccines and gene therapy constructs for animals and humans. One advantage to their use in vaccines is that adenoviruses are potent inducers of interferon, which may act as an adjuvant.[148] The replication-defective hAd5 construct used in FMD vaccine development is a live vector that lacks three regions of the adenovirus genome necessary for virus replication.[2] As a result, it cannot produce new adenoviruses except *in vitro*, in cells that carry the E1, E3 and E4 complementation functions (e.g., 293-ORF6 cells).[149,150] When a

vaccine construct is transfected into a packaging cell line that contains these functions, the cell generates virus-like particles consisting of the DNA vector inside an adenovirus capsid. These particles are able to attach to the cells of a number of species and become internalized;[151-153 cited in 149] however, they cannot replicate and infect additional cells. After it enters the cell, the vaccine construct is transported to the nucleus and transcribed to produce mRNA for vaccine proteins. The hAd5 vector does not integrate into the host genome, and the expression of vaccine proteins is transient.

The replication-defective hAd5-vectored FMD vaccines being developed in the U.S. contain the sequence for the P1-2A precursor (which encodes the complete FMDV capsid coding sequences and the 2A NSP) and the 3C NSP, a protease necessary for capsid assembly.[2,3,149,154-157] These constructs are expected to generate "empty capsids" of FMDV, without infectious FMDV nucleic acids, inside the cell. Unpublished studies have confirmed the production of empty capsids of approximately the same size as a native FMDV particle.[157] Some newer constructs also contain the full length sequence for the NSP 2B (previous constructs contained partial 2B sequences).[4] The partial or complete absence of coding sequences for some other NSPs allows these vaccines to be used with some NSP DIVA tests.

Live vectors can theoretically induce humoral responses, CMI and mucosal immunity, provided that all other factors (e.g., the route of administration) are appropriate. A recent study suggests that the replication-defective hAd5-vectored FMD construct, without 2B, protects pigs mainly by stimulating humoral immunity, although it also seems to induce minimal cytotoxic T cell responses.[20] Whether a more potent construct containing 2B[4] might result in higher levels of CMI is unknown. The equivalent experiments have not yet been conducted in cattle. There is no published evidence that hAd5-vectored replication defective FMD vaccines induce mucosal immunity after parenteral inoculation.

The hAd5 vaccine platform being developed in the U.S. is intended for the production of a variety of FMDV strains and serotypes; however, most research, to date, has used a construct that encodes the serotype A_{24} Cruzeiro capsid proteins.[2,4,57,149,154,157-160] This hAd5-vectored A_{24} vaccine has been granted a conditional license by the USDA CVB.[161] Including published and unpublished experiments, it has been tested in more than 300 cattle[57,157 and personal communication from personnel at DHS] and in a smaller number of pigs.[4,149,154,159,160] In addition to protecting animals from clinical signs, this vaccine prevented transmission between cattle in one study.[157] Other serotypes and subtypes of hAd5-vectored FMD vaccine candidates are reported to be in development for conditional and full USDA licensing programs.[157 and personal communication from personnel at DHS]

5.1 Desirable vaccine characteristics

5.1.1 Rapid onset of immunity

5.1.1.1 Cattle

Challenge studies suggest that the hAd5-vectored A_{24} vaccine can protect cattle as soon as 7 days after inoculation with a single dose.[157,158] In one study, 5 animals challenged after 7 days by intradermolingual inoculation were protected from disseminated disease and viremia, although one animal had a dental pad lesion without fever.[158] There was, however, evidence for some virus replication in the challenged cattle. A recent review article described several additional, previously unpublished, experiments. In one of these studies, 18 vaccinated cattle were all protected from clinical signs after intradermolingual challenge on day 7.[157] A second study evaluated animals that were challenged by contact with FMDV-infected cattle, 7 days after vaccination.[157] In this experiment, 4 of 6 cattle receiving the highest vaccine dose were completely protected from clinical signs, one had lesions only on the tongue, and the fifth animal developed generalized disease. Five of 6 cattle that received an intermediate dose of vaccine were completely protected, and the remaining animal only had tongue lesions, while 2 animals in the lowest dose group were completely protected, 2 developed tongue lesions alone, and 2 had generalized disease. All but two cattle, both in the lowest vaccine dose group, had detectable antibodies to FMDV by one week. The third group of experiments suggests that the hAD5-vectored A_{24} vaccine can reduce or eliminate transmission, when cattle are exposed to FMDV a week after immunization.[157] In this study, two groups of 3 vaccinated cattle were exposed to FMDV-infected cattle, for either 2 or 3 days. When the vaccinated animals were removed and placed in two separate rooms with vaccinated or nonvaccinated FMDV-naive cattle, there was no evidence for virus transmission. A single air sample from one room was weakly positive, on a single day; no virus was detected in the other room.

Additional vaccine candidates for other major serotypes and subtypes have reportedly shown high levels of protection in unpublished experimental challenge feasibility studies in cattle (personal communication from personnel at DHS). These animals were challenged two weeks after vaccination.

5.1.1.2 Pigs

Studies in pigs also suggest that they may be protected as soon as 7 days after immunization with the hAD5-vectored A_{24} vaccine, depending on the vaccine dose. In

one experiment, pigs given a single vaccine dose of 5 x 10^9 pfu and challenged 7, 14 or 42 days later were completely protected from clinical signs, and FMDV was not isolated from the blood or nasal swabs.[154] Antibodies to the 3D NSP were not detected, suggesting that virus replication did not occur, or occurred at only low levels. Neutralizing antibody titers to FMDV developed 1-2 weeks after vaccination, and remained stable for at least 8 weeks.[154] These titers were lower than the antibody titers induced by a commercial inactivated vaccine during the first week after immunization; however, they were equivalent by 14 days.[154] Pigs given a single, lower dose of vaccine (1 x 10^8 pfu) in a previous experiment also developed neutralizing antibodies within 2 weeks, but the titers decreased by 6 weeks unless the vaccination was boosted.[159] These animals were only partially protected from challenge at 8 weeks. (Pigs that received 2 doses were completely protected.)

The inclusion of the complete NSP 2B protein appears to increase the efficacy of hAd5-vectored FMD vaccines, and might result in a more rapid onset of protection. In a recent study, which used a construct that included the full length coding sequence for 2B and an optimized promoter, neutralizing antibodies to FMDV could be detected after 4 days.[4] Seven days after vaccination, antibody titers were significantly higher than in animals that received the construct without 2B. The titers decreased after 2 weeks and were comparable to vaccination without 2B by 3 weeks, at which time the pigs were challenged.

5.1.2 Long duration of immunity

Information on the DOI for the hAd5-vectored A$_{24}$ vaccine has not been published, although a single dose induced antibodies lasting at least 8 weeks in pigs.[154] However, in the field safety trial for vaccine licensing, an analysis of serum samples from a portion of the vaccinated dairy and beef cattle showed that 88% of the tested animals had detectable neutralizing antibodies to FMDV, approximately 12 months after they received a single vaccine dose (personal communication from personnel at DHS). A 6 month DOI study of this vaccine is expected to begin in the near future (personal communication from personnel at DHS).

5.1.3 Absence of interference with booster doses and other vaccines

Adenoviruses are widespread among animals, as well as people; however, human adenoviruses are thought to be sufficiently distinct that livestock would not have pre-existing immunity to hAd5-vectored vaccines.[149] Recent studies examining CMI and neutralizing antibody responses to human, bovine and porcine adenoviruses support

the absence of significant cross-reactivity between these viruses, although the possibility that cross-reactive, non-neutralizing antibodies might limit efficacy has not yet been ruled out.[162,163]

Immune responses to the hAd5 vector might limit a vaccine's efficacy if there is pre-existing immunity to other hAd5-vectored vaccines, or if multiple doses must be given.[148] Several studies have detected antibodies to the hAd5 vector in cattle and pigs immunized with hAD5-vectored FMD vaccines.[154,157,159,164] In one study, pigs that received a single dose (1 x 10^8 pfu) of the serotype A_{24} vaccine developed low titers to the vector, which peaked around week 4.[159] The titers were boosted in animals given 2 doses of the vaccine. Pigs that received a higher vaccine dose (5 x 10^9 pfu), developed anti-vector antibodies one week after vaccination, and these titers remained stable for 8 weeks. Deliberate induction of anti-vector antibodies, 2 weeks before pigs were immunized, decreased vaccine efficacy. These animals had lower neutralizing antibody titers to FMDV, and they were only partially protected from clinical signs after challenge. In cattle, titers to the hAd5 vector are reported to have a tendency to peak 2 weeks after vaccination, and a second dose of the A_{24} vaccine, given after the titers had declined, was able to boost the immune response.[2] Details of the latter experiment are currently unavailable.

5.1.4 Effectiveness in the presence of maternal antibody

There is currently no information about the effectiveness of hAd5-vectored FMD vaccines in the presence of maternal antibodies to FMDV. However, pigs were successfully vaccinated against swine influenza virus (SIV), in the presence of maternal antibodies to SIV, by priming with an hAd5-vectored swine influenza vaccine, then boosting with a commercial vaccine.[165]

5.1.5 Broad protection within serotypes

There is no information about cross-reactive immunity induced by hAd5-vectored FMD vaccines in pigs or ruminants. It is unlikely that these vaccines would be protective against other serotypes, but likely that they would provide some protection against other strains within a serotype. As with conventional inactivated vaccines, the degree of protection would probably be greater within some serotypes (e.g., serotype O) than others (e.g., serotype A).

5.1.6 Demonstration of safety and efficacy in multiple species (cattle, swine, sheep, goats)

The virus-like particles produced by packaging hAd5-vectored constructs can bind to and enter the cells of a number of species, including pigs and cattle.[151-153 cited in 149] Safety and efficacy studies with the hAd5-vectored serotype A_{24} vaccine have been conducted in cattle and swine.[157] Safety and efficacy studies with this construct are also planned in small ruminants.[157]

5.1.7 Knowledge of level of herd immunity needed to stop transmission in a population (Reproduction ratio)

One transmission experiment has been published for the hAd5-vectored A_{24} vaccine. These studies, which were described in a recent review article, reported that FMDV transmission did not occur between two small groups of vaccinated cattle, and transmission from vaccinated cattle to nonvaccinated cattle was not observed (for details, see section 5.1.1).[157] No transmission studies have been published in pigs, but hAd5-vectored A_{24} vaccines reduced virus shedding in some experiments.[4,154] There are no estimates of R, or estimates of the level of herd immunity needed to stop transmission with this vaccine.

5.2 Desirable characteristics for vaccine manufacture and stockpiling

5.2.1 Safety for manufacturing under BL2 conditions in the U.S.

No live FMDV is involved in the manufacture of hAd5-vectored FMD viruses, and high biological containment facilities are not needed.[57] Experimental batches of these vaccine candidates are already being made on the U.S. mainland under BL2 conditions.

5.2.2 Vaccine organism is not a select agent

Neither human adenovirus 5, which is a common respiratory virus in humans, nor the hAd5 vaccine vector are considered to be select agents.[122]

5.2.3 Capability of meeting 9 CFR regulatory requirements for purity, potency, safety, and efficacy

Initial studies of safety and efficacy have allowed the hAd5-vectored A_{24} Cruzeiro vaccine to be conditionally licensed by USDA CVB for use in cattle in the U.S.[161] Completed steps include production and characterization of a master seed virus, master cell line production and characterization, and the establishment of a scalable

manufacturing process for vaccine production.[157] This manufacturing process uses serum-free medium for suspension culture, together with a streamlined purification process, and produces vectors that are reproducible and of consistent quality and yield in each batch.[157] Genetic stability of the construct was demonstrated by the absence of sequence changes in both the FMDV insert and the vector after 10 serial passages.[157] In experiments described in a recent review article, 18 cattle vaccinated with increasingly purified hAd5-vectored A_{24} preparations from this manufacturing process were all protected from generalized disease after challenge, 7 days after vaccination.[157] In additional vaccine licensing studies using larger numbers of vaccinates, a very high level of protection was obtained following challenge at two weeks post-vaccination (personal communication from personnel at DHS).

Other completed steps include technology transfer to a CVB licensed manufacturing facility; the receipt of regulatory approval for an outline of production; the submission of Summary Information Format (SIF) and risk assessment to regulatory authorities; and the production of pre-licensing serials.[157] A large field safety study in beef and dairy cattle was completed as the final step prior to issuance of the conditional license (personal communication from personnel at DHS).

Additional safety and efficacy studies in swine and small ruminants (sheep and goats), need to be conducted.[157] *In vitro* tests, as an alternative to the PD_{50} potency test (which requires challenge with live virus and cannot be conducted on the U.S. mainland), are being investigated for hAd5-vectored FMD vaccine lot release.[166]

5,2.4 Capability of meeting requirements for cost effective manufacturing and all proprietary rights to vaccine antigen, vectors, and/or adjuvants

The conditionally licensed hAd5-vectored A_{24} Cruzeiro vaccine is capable of meeting all proprietary rights to the vaccine components. This vaccine is formulated with regulatory approved proprietary adjuvant, and it was prepared and released in accordance with USDA traditional vaccine programs and conditions (personal communication from personnel at Merial). The cost of manufacture is currently a concern, but this is not unexpected for a newly adopted platform, and would be expected to become more affordable over time (personal communication from personnel at Merial).

5.2.5 Desirable characteristics for stockpiling for emergency use

5.2.5.1 Ongoing manufacture and sale in endemic countries which enables indefinite delivery/indefinite quantity (IDIQ) contracts for just in time delivery

Currently, there are no active contracts for ongoing production of hAd5-vectored FMD vaccines for the U.S. in endemic countries. These vaccines are suitable for production in the U.S. In addition to emergency use in the U.S., hAd5-vectored FMD vaccines might be developed for vaccine markets outside the U.S.,[157] provided they are cost-effective, and the absence of interference between valences and the ability to obtain sufficient response after booster vaccination are demonstrated.

5.2.5.2 Stability when stored as bulk antigen

Human Ad5-vectored FMD vaccines are made as ready-to-use products.[2]

5.2.5.3 Long shelf-life and stability of finished vaccine

Preliminary studies suggest that hAd5-vectored FMD vaccines will be very stable for years in the frozen state.[157] A real-time stability program is ongoing, and it is expected that the shelf life, when stored frozen, will be at least 3 years (personal communication from personnel at DHS).

These vaccines are likely to be potent for several weeks if they are thawed and stored under refrigeration temperatures, or for several days under ambient temperatures.[157] Potency does not appear to be significantly affected by repeated freeze-thaw cycles.[157]

5.2.5.4 Rapid scale up and manufacture in the US in an emergency

There are currently no estimates on the speed of vaccine scale-up and manufacture, for hAd5-vectored FMD vaccines.

5.2.6 Rapid conversion of manufacturing to outbreak strain

The conditionally licensed hAd5-vectored FMD vaccine contains the P1-2A coding sequence for serotype A_{24} Cruzeiro.[154,157,159] The genetic sequence for the capsid of another strain could readily be inserted into this construct without adaptation to cell culture.[2,57] This should theoretically produce an effective vaccine for other FMDV serotypes and strains.[157] In practice, however, some of these constructs might be less effective than the A_{24} vaccine, and their efficacy should be validated.

Early experiments with serotype O vaccines (which require higher antigen doses in conventional vaccines[2,18,55,56]) did not demonstrate sufficient protection in pigs.[155,167] An

hAd5-vectored O_1 Campos vaccine provided only partial protection from challenge in these animals, even with the addition of GM-CSF as an adjuvant.[167] Furthermore, pigs vaccinated with a bivalent vaccine (A_{24} Cruzeiro and O_1 Campos) produced neutralizing antibodies against both serotypes, but the antibody titers were much lower than titers induced by either conventional commercial FMD vaccines or a monovalent hAd5-A_{24} vaccine in previous experiments.[155] It is possible that altered constructs may induce better immunity. The inclusion of the 2B protein in an hAd5-vectored O_1 Campos vaccine was reported to improve protection in challenged cattle (manuscript in preparation [cited in 157]). The inclusion of novel cell binding motifs into the hAd5 vector backbone has also resulted in improved efficacy (personal communication from personnel at DHS). Some new constructs based on the backbone used in the U.S. appear to be promising. In preliminary dose titration studies in cattle, an hAd5 serotype O vaccine candidate conferred good protection at same minimal protective dose identified for the conditionally licensed A_{24} Cruzeiro product (personal communication from personnel at DHS). Cattle efficacy feasibility studies using P1-2A coding sequences from more than ten different serotypes/subtypes have had excellent results (personal communication from personnel at DHS).

Full evaluation of each hAd5 construct for U.S regulatory approval would be expected to take 3-5 years[2] unless a conditional license is issued. Recent openness in the consideration of licensing vaccine platforms on the part of the USDA CVB, while not directly applicable, indicates a willingness to accept the attributes of a vaccine expressed in a backbone with established safety profiles such as hAd5 (personal communication from personnel at Merial).

5.3 Desirable characteristics for vaccine administration

5.3.1 Cost effective and practical delivery methods
The hAd5-vectored FMD vaccines are administered by intramuscular inoculation, similarly to conventional inactivated vaccines. Other routes, including subcutaneous administration (personal communication from personnel at DHS) and transdermal injection with needle free devices,[157] are being investigated. The decreased necessity for a cold chain (section 5.2.5.3), compared to inactivated FMD vaccines, could simplify administration during a vaccine campaign. A recent review article states that these vaccines are likely to be potent for several days under ambient temperatures.[157]

5.3.2 Safety for humans accidentally exposed to the vaccine

The hAd5 vector is based on human adenovirus 5, but it does not contain the structural genes for this virus and it is not replication competent. In the unlikely event of homologous recombination between an hAd5-vectored FMD vaccine and a wild type adenovirus, the result would be a replication-competent human adenovirus 5 without FMDV genetic material.[149,150] Because exposure to adenoviruses is common among children, the presence of such a construct in the environment is not expected to be a concern.[149,150] Wild type human adenovirus 5 causes mild, self-limiting illness or inapparent infections in immunocompetent individuals.[147] In children, it is an important cause of mild respiratory disease with cold-like symptoms. Conjunctivitis has been reported after experimental inoculation of the eye in human volunteers.

Adenoviral constructs and adenoviruses have been tested or used in humans for a number of years. In people, live non-attenuated adenoviruses are given orally as vaccines against adenovirus-mediated respiratory disease.[147] Vaccines containing human adenoviruses 4, 7 and 21 have been used for this purpose for many years, and have an excellent safety record. Live human adenovirus 5 vaccines have also been tested by enteric administration, without adverse effects. Trials with various hAd5-vectored constructs, administered by parenteral routes, have been conducted in people.[147] Concerns about the parenteral use of these constructs have mainly been associated with human cancer treatment and gene-therapy trials, especially when these agents are administered intravenously at higher doses.[147,168] Adenoviruses are very effective inducers of interferon and innate immune responses, and these responses can result in unexpected adverse effects.[148] Nevertheless, conditionally replicating adenoviruses have been used in phase I and phase II clinical trials in cancer patients, with only mild clinical signs such as flu-like symptoms and injection site pain, when they are injected directly into the tumor or administered intraperitoneally.[168]

5.4 Desirable characteristics for minimizing the impact on food production and animal trade

5.4.1 Safety for use in food producing animals with no, or reasonably short, withdrawal time for animal products for human consumption

5.4.1.1 Absence of replication competent viruses, reversion to virulence and extraneous pathogens

The generation of replication-competent viruses can be an issue with some viral-vectored vaccines. Theoretical considerations suggest that this will not be a concern for hAd5-vectored FMD constructs. In these constructs, the FMDV viral sequences are cloned into the essential E1 region of the adenovirus genome.[149] Homologous

recombination with a wild type adenovirus is unlikely to occur, and if it did, it would produce a replication-competent human adenovirus without FMDV genetic material.[149,150] Wild type human adenovirus 5 causes illness in people,[147] and is not a health concern in livestock.

Testing for reversion to virulence (backpassage studies, administered by the route most likely to result in reisolation of the vaccine virus) and shed-spread into the environment (administered by the route intended for the product's use) were conducted for the hAd5-vectored FMD A$_{24}$ vaccine, as part of the risk assessment in vaccine licensing studies.[157] Because human adenovirus 5 is a respiratory pathogen of people, intranasal inoculation of the master seed vaccine virus was used in studies to demonstrate the absence of reversion to virulence.[157] The virus was not isolated from the nasal passages or oral samples of calves for 14 days, with the exception of the nasal passages during the initial 24 hours after administration. In shed-spread studies, healthy cattle and pigs were inoculated intramuscularly with the vaccine.[157] There was no evidence of vaccine transmission to naive cattle or pigs in contact, based on the absence of humoral immune responses to either FMDV or the hAd5 vector, and the vaccine virus was not isolated from these animals.[157]

All master seeds accepted by the USDA will meet the same requirements as all federally licensed products, and the conditionally licensed hAd5-vectored A$_{24}$ Cruzeiro vaccine and all future seeds will meet these standards (personal communication from personnel at Merial). As with other vaccines,[17] MSVs and MCSs are tested during manufacture for freedom from contaminating microorganisms including mycoplasma, bacteria, fungi and viruses. Starting materials of biological origin are usually tested for the absence of adventitious agents or obtained as gamma irradiated products.

5.4.1.2 Allergic reactions or other adverse reactions in vaccinated animals

In one reported study, no local or systemic reactions were reported during the first 72 hours, in 18 cattle immunized with the hAd5-vectored FMD A$_{24}$ vaccine.[157] Additional studies in cattle have shown an excellent safety profile (personal communication from personnel at DHS). Safety studies in swine and small ruminants (sheep and goats) are still required.[157]

5.4.1.3 Withdrawal period and safety for human consumption

Due to regulatory requirements, all vaccines for food animals in the U.S. must be labeled with a minimum slaughter withdrawal time of 21 days. A withdrawal time of 21 days might be feasible for the conditionally licensed hAd5-vectored FMD vaccine, which does not include an adjuvant that would cause local inflammation. However, this is still under consideration at this time (personal communication from personnel at the Department for Homeland Security).

5.4.3 Availability of companion diagnostic test to detect infections in vaccinated animals (DIVA)

The hAd5-vectored FMD vaccine platform contains only the capsid proteins and the nonstructural proteins 2A, 3C, and in some cases 2B.[2-4,149,154-157] Because most NSPs are absent, hAd5-vectored FMD vaccines can be used with a variety of DIVA tests including the 3ABC ELISA.[2,3 and personal communication from personnel at DHS] Although the full length 3C protein is encoded by this vaccine, seroconversion does not seem to occur in this test (personal communication from personnel at DHS). Rare false positives have been identified among animals; however, these animals were seropositive before vaccination (personal communication from personnel at DHS).

DIVA capability would be expected to allow hAd5-vectored FMD vaccines to be used with surveillance programs intended to document freedom from infection for international trade. However, this will require validation of the DIVA assays for this purpose.

5.5 Desirable characteristics for controlling FMD in wild and feral populations

5.5.1 Safety and efficacy when delivered orally in baits for feral swine or deer

Oral vaccines are challenging to develop,[137,138] and no published studies have described such vaccines for FMD. The successful development of hAd5-vectored oral rabies vaccines for wildlife suggests that, theoretically, this system might also be used to develop oral FMD vaccines. There is currently very little information on the induction of mucosal responses by these vaccines. Intranasal inoculation of one hAd5-vectored, replication competent, serotype C FMDV construct (without NSPs) did not induce significant mucosal responses in early experiments; however, this was attributed to poor replication of the construct at this site.[164]

6. ALPHAVIRUS-VECTORED FMD VACCINES

Alphaviruses (family Togaviridae) are enveloped viruses with positive sense, single-stranded RNA genomes.[169,170] "Replicon" vectors have been developed from members of this family of viruses. These vectors essentially duplicate the early steps after an alphavirus enters a cell, to produce vaccine proteins rather than alphavirus structural proteins. Alphavirus replicon constructs contain a highly active alphaviral RNA promoter, which drives the expression of the inserted gene(s), together with the replication elements needed for amplification of the RNA construct.[169,171] The constructs replicate in the cytoplasm of infected cells, and can provide high levels of antigen expression.[169-172] In some cells, replicon vectors may produce approximately 200,000 copies of themselves, and the expressed protein may comprise up to 15-20% of the total cellular protein.[170,173]

Because the alphavirus structural proteins have been removed, replicon vectors are incapable of generating new virions and spreading to other cells.[169,170,172,174] The vectors are usually delivered to the cell by packaging the vector construct into virus-like particles and using natural receptor-mediated entry.[169] Packaging can be accomplished by transiently co-transfecting the *in vitro* transcribed replicon vector with one or more helper RNAs that code for the alphavirus structural proteins.[169,170] The helper RNA does not contain the encapsidation sequences that would be used to package it into the capsid in nature.[170] Alternatively, replicon vectors can be introduced into packaging cell lines, which express the structural proteins from DNA cassettes.[169]

Replicon particles can target particular cell types, depending on the structural coat proteins used for packaging.[169] The three major alphavirus replicon vectors used in vaccines have been generated from the Sindbis virus, Semliki Forest virus and Venezuelan equine encephalitis virus (VEE) genomes.[169,171] The VEE vector, which is used for the replicon particle (RP) FMD vaccine, is based on the TC-83 vaccine strain of this organism.[175] The natural tropism of the VEE virion may result in a particularly efficient vaccination response.[176] During an infection, this virus targets dendritic cells, which migrate to the draining lymph node, where the virus then replicates.[172] Interestingly, "empty" VEE replicon particles, which do not express an antigen gene, can also act as adjuvants for the induction of humoral, cellular and mucosal immunity to co-delivered antigens.[177-179] These particles appear to transiently induce an inflammatory environment in the draining lymph node, and upregulate inflammatory cytokines and chemokines.[177]

RP FMD vaccines in development in the U.S. are intended for the production of a variety of viral strains and serotypes. To date, two vaccine candidates have been constructed.[180]

<superscript>and personal communication from personnel at Harrisvaccines</superscript> The first vaccine encodes the capsid and 3C coding regions of the A_{24} Cruzeiro strain of FMDV.[180] Information presented at a recent conference, and additional unpublished research, suggests that this vaccine can induce humoral immunity and protects cattle from challenge.[180] and personal communication from personnel at DHS It is also reported to induce neutralizing antibodies in swine (personal communication from personnel at Harrisvaccines). There is no information about whether RP FMD vaccines stimulate other forms of immunity; however, alphavirus replicon particles encoding other antigens have been found to induce cellular and mucosal immune responses in various host species.[169,171,176,178] Under some conditions, mucosal responses have resulted from parenteral vaccination.[169,177,178,178] A second RP FMD vaccine that contains serotype SAT-2 has also been constructed, and tested in two challenge studies in cattle and an immunogenicity trial in pigs (personal communication from personnel at Harrisvaccines and DHS).

6.1 Desirable vaccine characteristics

6.1.1 Rapid onset of immunity

Protection from challenge, using the RP serotype A_{24} Cruzeiro FMD vaccine, has been examined 2 and 3 weeks after vaccination, although antibody responses suggest the possibility of some earlier protection. In the initial study described at a recent conference, a group of 4 cattle that received 1×10^9 replicon particles all had detectable neutralizing antibodies by 7 days, and all but one animal were completely protected from clinical signs when they were challenged after 3 weeks.[180] In a second, unpublished study using the same vaccine dose, cattle were also protected when they were challenged at 2 weeks (personal communication from personnel at Harrisvaccines). A lower vaccine dose was less effective: only half of the 4 animals that received 5×10^8 replicon particles had neutralizing antibodies 2 weeks after vaccination, and partial protection was seen after challenge at 3 weeks.[180] The company also reports successful cattle challenge experiments after immunization with a SAT-2 vaccine; however, details are not currently available, pending publication (personal communication from personnel at Harrisvaccines).

There are no challenge studies yet in swine. In a preliminary swine immunogenicity study (n=2 animals), neutralizing antibodies were detected in both animals 6 days after vaccination with RP serotype A_{24} Cruzeiro FMD vaccine (personal communication from personnel at Harrisvaccines). In a similar study using a RP serotype SAT-2 vaccine, neutralizing antibodies were detected in two pigs as early as 7 days (personal communication from personnel at Harrisvaccines).

6.1.2 Long duration of immunity

The DOI for RP FMD vaccines is not yet known. In guinea pigs and mice, alphavirus replicon vaccines for other pathogens have resulted in immunity lasting up to a year (personal communication from personnel at Harrisvaccines). The DOI is likely to vary with both the pathogen and the host species, and a literature search revealed no DOI studies for any alphavirus replicon vaccine in pigs, cattle or small ruminants.

6.1.3 Absence of interference with booster doses and other vaccines

Most animal populations do not have pre-existing immunity to VEE,[176] making interference with the initial vaccination unlikely. Whether immune responses to the VEE replicon vector could affect subsequent boosters is still under investigation.[174,177] Theoretically, minimal anti-vector responses would be expected after vaccination, as only small amounts of the capsid are injected into the animal, and the capsid proteins are not expressed during virus replication.[170] However, anti-vector (VEE) antibodies have been reported in mice, nonhuman primates and people, particularly after repeated administration of a vaccine.[174,181-183] In one clinical trial in healthy human volunteers, antibodies specific for the VEE vector began to develop after the first vaccine dose, with titers rising to ≥ 1:160 after 2 doses in some recipients, and reaching 1:320 or higher in all recipients after 3 doses.[181] In another human study, the geometric mean titers of anti-vector antibodies were dose dependent, and ranged from 10 to 4159.[182]

No published studies have investigated the extent to which anti-vector responses interfere with subsequent doses of alphavirus-vectored vaccines in livestock, but reports from mice,[173,174,176] nonhuman primates[183,184] and people,[181] as well as an unpublished study in ferrets,[170] suggest that significant boosting may occur despite such responses. However, the response to both the vector and the expressed antigen may be affected by the species of animal and the route of inoculation,[173] and the effects of anti-vector immunity have not yet been described in ruminants or pigs. In the event of interference by anti-vector responses, boosting with the antigen alone might be feasible.[177]

6.1.3 Effectiveness in the presence of maternal antibody

There is currently no information regarding the effectiveness of RP FMD vaccines in the presence of maternal antibodies to FMDV, and reports based on other alphavirus-based vaccines are contradictory. Although VEE-vectored replicon vaccines can immunize mice against dengue virus in the presence of maternal antibodies to this virus,[185] an

alphavirus replicon vaccine for swine influenza was ineffective in pigs with maternal antibodies to SIV.[186]

6.1.4 Broad protection within serotypes

There is no information about cross-reactive immunity induced by RP FMD vaccines. It is unlikely that these vaccines would be protective against other serotypes, but likely that they would provide some protection against other strains within a serotype. As with conventional inactivated vaccines, the degree of protection would probably be greater within some serotypes (e.g., serotype O) than others (e.g., serotype A).

6.1.5 Demonstration of safety and efficacy in multiple species (cattle, swine, sheep, goats)

RP FMD vaccines expressing antigens for serotypes A_{24} Cruzeiro and SAT-2 have been tested in cattle (challenge trials) and pigs (preliminary immunogenicity studies).[180 and personal communication from personnel at Harrisvaccines] VEE replicon vaccines encoding other antigens have been investigated in a number of animal species including pigs, mice, rabbits, cats, horses, chickens, guinea pigs and nonhuman primates, as well as in people.[175,178,186-188] In the U.S., an RP swine influenza vaccine has been licensed for use in pigs (personal communication from personnel at Harrisvaccines).

6.1.6 Knowledge of level of herd immunity needed to stop transmission in a population (Reproduction ratio)

No transmission studies have been published yet for the RP FMD vaccines. There are no estimates of the level of herd immunity needed to stop transmission with any RP FMD vaccine.

6.2 Desirable characteristics for vaccine manufacture and stockpiling

6.2.1 Safety for manufacturing under BL2 conditions in the U.S.

Alphavirus replicon vaccines can be manufactured without growing live FMDV, and high biosafety level facilities are not necessary for vaccine production.[170] Experimental batches of the RP FMD vaccine (serotypes A_{24} Cruzeiro and SAT-2) are being made in the U.S. under BL2 conditions (personal communication from personnel at Harrisvaccines).

6.2.2 Vaccine organism is not a select agent

Although VEEV is listed by the U.S. government as a select agent,[122] the TC-83 vaccine strain used in alphavirus replicon technology is specifically excluded from this list in the most recent Biosafety in Microbiological and Biomedical Laboratories (BMBL) manual published by the NIH and CDC.

6.2.3 Capability of meeting 9 CFR regulatory requirements for purity, potency, safety, and efficacy

The RP serotypes A_{24} Cruzeiro and SAT-2 FMD vaccines are still in the early stages of development. RP FMD vaccines should be capable of meeting 9 CFR regulatory requirements for purity and safety, as another VEE-vectored replicon vaccine (for H3N2 swine influenza virus) from the same company has been approved for use in pigs in the U.S. (personal communication from personnel at Harrisvaccines). APHIS issued an Environmental Assessment and Finding of "no significant impact" for this system (personal communication from personnel at Harrisvaccines). Potency and efficacy for the two initial RP FMD vaccines remain to be established, but early experiments appear promising.[180 and personal communication from personnel at Harrisvaccines and DHS]

6.2.4 Capability of meeting requirements for cost effective manufacturing and all proprietary rights to vaccine antigen, vectors, and/or adjuvants

Harrisvaccines, which is developing the RP FMD vaccines, has an exclusive license for the global rights to develop and commercialize vaccines against diseases of swine and ruminants, using the unique RP platform technology (personal communication from personnel at Harrisvaccines). The company also states that production is low cost and scalable (personal communication from personnel at Harrisvaccines).

6.2.5 Desirable characteristics for stockpiling for emergency use

6.2.5.1 Ongoing manufacture and sale in endemic countries which enables indefinite delivery/indefinite quantity (IDIQ) contracts for just in time delivery

Currently, there do not appear to be any contracts to produce RP FMD vaccines for use in endemic countries. These vaccines are suitable for production in the U.S.

6.2.5.2 Stability when stored as bulk antigen

Alphavirus replicon particles are stable when stored frozen as bulk vaccines (personal communication from personnel at Harrisvaccines).

6.5.2.3 Long shelf-life and stability of finished vaccine

Formulated liquid RP vaccines are stable for at least two months with refrigeration (personal communication from personnel at Harrisvaccines).

6.5.2.4 Rapid scale up and manufacture in the US in an emergency

At present, Harrisvaccines is able to manufacture millions of doses of a replicon particle vaccine a month, and production is scalable (personal communication from personnel at Harrisvaccines).

6.5.3 Rapid conversion of manufacturing to outbreak strain

It would be possible to convert an RP FMD vaccine construct rapidly to another strain. The coding sequence for any FMDV capsid could be inserted into this construct without adaptation to culture.[175] Rapid conversion to a field strain has been demonstrated for influenza viruses in the RP system: a novel vaccine was produced 51 days after the virus genome sequence became available.[189 and personal communication from personnel at Harrisvaccines] This process included the synthesis of the influenza H1 gene, cloning of the gene into the replicon vector system, optimization of H1 RP protein expression and RP yields, production of the vaccine for animal studies, and performance of studies characterizing immune responses in vaccinated animals.

Currently, RP FMD vaccine candidates containing two different serotypes (A_{24} Cruzeiro and an unspecified SAT-2 strain) have been shown to protect cattle from clinical signs after challenge.[180 and personal communication from personnel at Harrisvaccines and DHS] Both vaccines also induced neutralizing antibodies in preliminary studies in swine (personal communication from personnel at Harrisvaccines). It is, nevertheless, possible that potent vaccines for some serotypes (e.g., serotype O) or strains might be more difficult to construct. The ability of RP FMD vaccines to express high levels of proteins[170,173] could be an advantage for producing efficacious serotype O vaccines, which may require higher levels of antigen.[2,18,55,56]

Full evaluation of each construct for U.S regulatory approval would be expected to take 3-5 years[2] unless a conditional license is issued. Vaccines with new FMDV antigens might

become available sooner, if the RP FMD vaccines qualify for inclusion as a production platform under APHIS guidelines for licensing production platforms (Veterinary Services Memorandum 800.213).

6.6 Desirable characteristics for vaccine administration

6.6.1 Cost effective and practical delivery methods

At present, RP FMD vaccines are prepared as liquid or frozen products, and require a cold chain for shipping and storage (personal communication from personnel at Harrisvaccines). In initial tests, cattle vaccinated with the RP serotype A_{24} Cruzeiro FMD vaccine were immunized by intramuscular inoculation.[180] Other delivery methods have not yet been tested with RP FMD vaccines; however, intradermal and needle-free administration of RP influenza vaccines have been successful in swine and other species (personal communication from personnel at Harrisvaccines).

6.6.2 Safety for humans accidentally exposed to the vaccine

Alphavirus-vectored vaccines are still relatively new. However, several vaccines that encode viral or tumor antigens have been tested in phase I (safety) clinical trials in humans.[181,182,190,191] No serious adverse effects were attributed to these vaccines, although mild to moderate injection site reactions were seen in some studies, especially at higher doses and after subcutaneous inoculation.[181,182,190,191] In one clinical trial, injection site reactions persisted for 3 to 65 days after subcutaneous inoculation of an alphavirus-vectored cytomegalovirus vaccine.[181] Nausea, vomiting and mild to moderate myalgia were also attributed to this vaccine in some healthy human volunteers.[181]

The potential for generating replication-competent viruses during the packaging of RP vaccines is also a consideration. Although this was a problem with early packaging systems, several safeguards have greatly decreased the risk, and infectious viruses have not been recovered from newer systems at detectable levels (for details, see section 6.7.2.1).[170,172,173] If any recombinant viruses are produced, they should theoretically be no more virulent that the TC-83 vaccine strain upon which the VEE replicon vector is based.[170,175] In the U.S., live attenuated TC-83 is used as an Investigational New Drug (IND) to vaccinate some laboratory workers and the military against Venezuelan equine encephalitis. The safety of this vaccine for humans has been investigated in several reports. One study assessed adverse effects, over a 15-year period, in one laboratory where the vaccine is used.[192] There were no serious or permanent side effects during this time, although 23% of vaccinated personnel had self-limited flu-like reactions, which

ranged from mild signs that lasted a few hours and required no treatment, to illnesses that lasted 2-3 days and were treated with mild analgesics (e.g., ibuprofen) and bed rest. Similar side effects were reported in up to 40% of TC-83 vaccine recipients in other studies.[193] Live TC-83 vaccine is not administered to pregnant women, as possible teratogenic effects have been noted in animal studies,[194 cited in 192] and there is one anecdotal report of a human case that may have been linked to this vaccine.[195]

6.7 Desirable characteristics for minimizing the impact on food production and animal trade

6.7.1 Availability of companion diagnostic test to detect infections in vaccinated animals (DIVA)

The RP FMD vaccines in development code for the capsid and 3C coding regions of FMDV.[180] This construct allows the use of serological DIVA tests based on NSPs not included in the vaccine construct. The *PrioCHECK*® FMDV ELISA (Prionics AG, Switzerland) has been used to measure antibodies to FMDV 3ABC nonstructural proteins, according to manufacturer's instructions, on serum from A_{24} Cruzeiro RP vaccinated cattle (personal communication from personnel at Harrisvaccines). Vaccination did not induce serum antibodies to 3ABC in this NSP test, providing initial evidence that these are DIVA vaccine candidates.

DIVA capability would be expected to allow RP FMD vaccines to be used with surveillance programs intended to document freedom from infection for international trade. However, this will require validation of the DIVA assays for this purpose.

6.7.2 Safety for use in food producing animals with no, or reasonably short, withdrawal time for animal products for human consumption

6.7.2.1 Absence of replication competent viruses, reversion to virulence and extraneous pathogens

The risk of producing replication-competent alphaviruses depends on the system used to package these viruses. One method of packaging is to co-transfect the *in vitro* transcribed replicon vector with a helper RNA that codes for the structural proteins, but does not contain the alphavirus encapsidation sequences.[170] Replication-competent viruses were sometimes produced in early versions of this system, which used a single helper RNA that coded for all the viral proteins, and required only a single recombination event to generate an infectious virus.[169,170] A 'split helper' system, with the RNA for the structural proteins divided between two helper RNAs, has greatly decreased the probability of this event, as at least two recombination events are now

required.[170,172,173] Although it is still theoretically possible to produce a replication competent virus,[173] infectious viruses have not been recovered from such systems at measurable levels.[170,172,173] Additional safeguards that have recently been incorporated include the removal of promoter sequences from the helper RNAs and the insertion of a stop codon to eliminate the cleavage activity of the capsid protein [170] If any recombinant viruses are produced, they should theoretically be no more virulent that the TC-83 vaccine strain of the VEE virus, upon which the replicon vector is based.[170,175]

RP FMD vaccines are still in the initial stages of development; however, demonstration of the absence of reversion to virulence and shed-spread into the environment were completed for an RP swine influenza vaccine produced by the same company.[175] No evidence of vaccine shedding, transmission of the vaccine virus to nonvaccinated, co-housed animals, or reversion to virulence was detected in pigs or mice, using a 200-fold higher dose than that used in challenge studies.[175] APHIS issued an Environmental Assessment and Finding of "no significant impact" for this system (personal communication from personnel at Harrisvaccines).

The master cell stock (MCS) has been tested by CVB for freedom from contaminating microorganisms, and has been approved for use in the production of RP products for cattle, swine, goats and pigs, as well as some other species not susceptible to FMDV (personal communication from personnel at Harrisvaccines).

6.7.2.2 Allergic reactions or other adverse effects in vaccinated animals

Preliminary results have shown that there were no local or systemic reactions at the vaccine doses used in the initial studies (personal communication from personnel at DHS).

6.7.2.3 Withdrawal period and safety for human consumption

The RP serotype A_{24} Cruzeiro and SAT-2 FMD vaccine candidates do not require an adjuvant that would cause local inflammation. The USDA established a withdrawal period of 21 days for a swine influenza vaccine based on the same RP system and produced by the same company (personal communication from personnel at Harrisvaccines). A similar withdrawal period might be expected for RP FMD vaccines.

6.7.3 Availability of companion diagnostic test to detect infections in vaccinated animals (DIVA)

The RP FMD vaccines in development code for the capsid and 3C coding regions of FMDV.[180] This should allow the use of serological DIVA tests based on NSPs not included in the vaccine construct. Commercially available diagnostic tests (*PrioCHECK® FMDV ELISA, Prionics AG, Switzerland*) have been confirmed to be DIVA-compatible in RP FMD vaccinated cattle (personal communication from personnel at Harrisvaccines).

DIVA capability would be expected to allow RP FMD vaccines to be used with surveillance programs intended to document freedom from infection for international trade. However, this will require validation of the DIVA assays for this purpose.

6.8 Desirable characteristics for controlling FMD in wild and feral populations

6.8.1 Safety and efficacy when delivered orally in baits for feral swine or deer

There are no published reports on the development of alphavirus replicon vaccines as oral vaccines for FMD.

7. PLASMID DNA VACCINES

7.0.1 General principles

DNA vaccines consist of plasmids that encode the genes for vaccine proteins, together with the elements needed for gene transcription and the sequences required for plasmid replication during the manufacturing process in bacterial cell cultures.[196] Some vaccines code for optimized antigen sequences intended to target highly variable pathogens.[197] By incorporating only selected viral genetic sequences for specific proteins (e.g., FMDV capsid proteins), DNA vaccines can be used with DIVA tests. Additional elements may be included to enhance immunity or modulate the immune response.[197,198] They can include motifs recognized during the innate immune response, such as unmethylated CpG motifs; cytokines and chemokines incorporated as adjuvants (e.g., GM-CSF); or other components such as co-stimulatory molecules.[198] These elements may be engineered onto the same plasmid used to express the vaccine antigens, or placed on a separate plasmid included in the vaccine. Cytokines and chemokines in DNA vaccines have the advantage that they are expressed as long as the antigen.[198]

DNA vaccines are manufactured by growing plasmids in bacterial cells, then purifying and concentrating the plasmids from bacterial lysates.[199] When the vaccine is injected into an animal, some of the plasmids are taken up by cells and reach the nucleus. The genes they carry are transcribed and translated via cellular protein synthesis mechanisms. The cell types that take up plasmids vary with the location at which they are injected. After intramuscular administration, plasmids are found in resident antigen presenting cells and myocytes.[198,200] A challenge for DNA vaccination is that only a small proportion of the injected DNA is taken up by cells, and all of this DNA does not reach the nucleus.[169,200,201] Unprotected DNA outside the cell is degraded and quickly disappears.[169] The poor uptake of DNA is thought to be a major factor in the weak and short-lived immune responses induced by some vaccines, even when multiple doses are given.[64] One method to improve a vaccine's potency is to give a higher dose of plasmids.[169] Another is to improve DNA uptake by cells, using techniques such as electroporation, the use of particle bombardment ("gene gun") or high-pressure delivery, or the incorporation of the plasmid onto particles such as cationic liposomes or poly (d,l-lactide-co-glycolide) (PLG) particles.[64,169,196-198,202]

DNA vaccines mainly generate processed antigens on the surfaces of transfected cells.[57,198] Depending on the cell type, the antigens may be found in MHC I molecules alone, or in both MHC-I and MHC-II molecules.[57,198] As a result, DNA vaccines have the potential to stimulate a wide variety of immune responses including innate immunity, humoral immunity, CMI and mucosal responses.[16,196,201] However, generating an effective and balanced immune response can be difficult.[203] Although the mechanisms are still poorly understood, both the delivery method and the injection site seem to influence the type of immune response: some methods mainly result in CMI, while others tend to induce humoral responses.[196,200] The formulation of the vaccine was also reported to influence immune responses in sheep.[64] In these animals, incorporating an FMD DNA vaccine on cationic liposomes improved humoral responses compared to naked DNA, but seemed unable to induce CMI, while incorporating the vaccine on PLG particles resulted in better CMI. Mucosal delivery of DNA vaccines can induce both mucosal and systemic immunity, but parenteral inoculation does not necessarily result in mucosal responses.[200]

7.0.2 Promising approaches to DNA vaccination for FMD

Several reports have described complete clinical protection of pigs, cattle or sheep immunized with DNA vaccines.[196] It is, however, difficult to make generalizations about the optimal approach, as the successful techniques and constructs differed, and similar approaches were sometimes less effective in other studies.[196] In one successful

experiment, challenged pigs did not develop clinical signs after vaccination with a low dose of a DNA construct that contained only two epitopes (residues 141–160 and 200–213) from a serotype O FMDV capsid protein (1D/ VP1) carried on a self IgG molecule.[204] Delivery was by gene gun, and inoculation into the ear was protective, but inoculation into the thigh was not. This group reported that the construct also provided complete clinical protection when pigs received larger doses, the vaccine contained porcine IL-2, and it was administered by injection rather than gene gun.[205] Other studies, using similar but not identical approaches, or different DNA constructs, reported only partial clinical protection despite the induction of humoral immunity and CMI in some cases.[196] It is possible that inoculation into the ear was an important factor in the successful studies.[196]

Promising results were also reported in a series of experiments that vaccinated pigs, sheep or cattle with a plasmid encoding the FMDV serotype O_1 Kaufbeuren P1-2A, 3C and 3D regions, together with a plasmid for species-specific GM-CSF.[8,16,64,206,207,207] These studies illustrate the many variables that may influence the response to a DNA vaccine. In the initial experiments, pigs were clinically protected when the DNA constructs were injected simultaneously by both intramuscular inoculation and intradermally into the ears.[206] Pigs that did not receive GM-CSF were not protected. In a follow-up study, the same route of administration induced modest antibody titers to FMDV after an initial dose and two boosters, but there was little or no antibody response if the constructs were injected intramuscularly into a single site.[8] A prime-boost protocol, with one DNA vaccination into multiple sites, followed by boosting with inactivated FMD viruses and recombinant 3D protein, resulted in antibody titers similar to those induced by conventional inactivated vaccines.[16] Additional doses of the DNA vaccine before protein boosting resulted in much higher levels of neutralizing antibodies and complete protection of pigs from clinical signs.[16] Interestingly, this prime-boost protocol also induced cross-reactive antibodies to FMDV serotypes A, C and Asia1, and good antibody titers to O_1 Manisa and O_1 Lausanne as well as the vaccine strain O_1 Kaufbeuren.[16] A similar prime-boost protocol, with the incorporation of the DNA onto PLG particles, was protective in sheep; however, a vaccine that contained GM-CSF but did not use PLG particles was less successful in this species.[64] In cattle, intramuscular injection of a single dose of the DNA vaccine and GM-CSF plasmid, with electroporation, was a promising approach.[207] This protocol was ineffective without electroporation, and the incorporation of the plasmid on PLG particles did not improve immune responses. Cross-reactive neutralizing antibodies were induced to a serotype Asia 1 virus, but not to other serotypes.[207]

7.0.3 Inovio SynCon® FMD vaccines

Limited information regarding the FMD DNA vaccines being developed in the U.S. is available in a press release from the manufacturer, Inovio Pharmaceuticals, and its subsidiary, VGX Animal Health.[208] The initial study has apparently not been published in the scientific literature at this time. At the Vaccine World Summit in New Delhi, India (2011), the company reported that pigs vaccinated with a SynCon® universal FMD vaccine representing "four of the most common FMD serotypes" developed high antibody titers reactive to all 4 serotypes, with an additional increase after boosting.[208] Vaccinated animals were also reported to develop good T-cell responses; however, the type of antibody response and T cell response were not specified in the press release. The vaccine was administered by electroporation. Several FMD serotype-specific vaccine candidates are in the research stage in the U.S. (personal communication from personnel at DHS) In a second swine immunogenicity study using DNA constructs based on the P1 region from two different FMDV serotypes, both serum virus neutralizing titers and CMI responses were detected following two doses (personal communication from personnel at DHS).

7.1 Desirable vaccine characteristics

7.1.1 Rapid onset of immunity

There is no published information on the onset of immunity for the SynCon® FMD vaccines. In other approaches that used different protocols and vaccines, full clinical protection was reported after a minimum of 2 vaccine doses, or a DNA vaccine and a protein boost, resulting in a relatively prolonged vaccination process.[16,64,204-207] Once the immunization protocol was completed, animals were sometimes protected when they were challenged 7-10 days later.[16,64,196,204,205]

7.1.2 Long duration of immunity

Theoretically, DNA vaccines may induce long-lasting immunity. Mammalian cells expressing plasmid coded proteins can do so until they naturally turn over, at which time the DNA coding for the viral proteins is lost to normal cellular debris "clean up" mechanisms.[201 and personal communication from personnel at VGX Animal Health] Length of expression is therefore primarily dictated by the antigenicity of the protein being expressed, in concert with the transfected cell type's half-life. If an antigen is very immunogenic, and a strong CMI response is triggered, expression is maximized at 7-10 days post vaccination, and falls off precipitously by day 10 post vaccine administration and electroporation.[201 and personal communication from personnel at VGX Animal Health] Expression can,

however, be much longer. In various experiments, plasmids persisted in cells at the injection site for up to 4 weeks in pigs, more than 2 years in mice, at least 4 weeks in rats, and for 54 days in sheep, 10 weeks in turkeys and 70-90 days in fish.[200] The vaccine protein may be expressed as long as the plasmid persists, or expression may stop sooner.[200] There is no information on the DOI for the SynCon® FMD vaccines.

7.1.3 Absence of interference with booster doses and other vaccines

DNA vaccines can be administered repeatedly without interference from immune responses.[202] These vaccines can also be boosted by proteins (e.g., inactivated FMD vaccines), sometimes resulting in a more effective response.[16,207]

There was no evidence of vaccine boost interference in a swine immunogenicity study of SynCon® FMD vaccines (personal communication from personnel at DHS).

7.1.4 Effectiveness in the presence of maternal antibody

DNA vaccines have been reported to be effective in the presence of maternal antibodies.[196,203] However, there is no specific information for the SynCon® FMD vaccines or any other FMD DNA vaccine.

7.1.5 Broad protection within serotypes

In a study reported at the Vaccine World Summit in New Delhi, India (2011), pigs vaccinated with a SynCon® universal FMD vaccine that represented "four of the most common FMD serotypes" developed high antibody titers reactive to all 4 serotypes, with an additional increase after boosting. The company is attempting to develop a vaccine that generates broader, cross-reactive immune responses.[208]

7.1.6 Demonstration of safety and efficacy in multiple species (cattle, swine, sheep, goats)

SynCon® FMD vaccines have been tested in pigs, which were reported to develop good serological responses to FMDV.[208 and personal communication from personnel at DHS] There is no published information on challenge studies with these vaccines in pigs or other species.

Studies with other types of FMD DNA vaccines suggest that pigs, sheep and cattle can all be protected from challenge by some formulations.[8,16,64,196,204-206] In some cases, a protocol that was effective in one species required modification to protect a different species.[16,64,196,207]

7.1.7 Knowledge of level of herd immunity needed to stop transmission in a population (Reproduction ratio)

No transmission studies have been published yet for the SynCon® FMD vaccines. There are no estimates of the level of herd immunity needed to stop transmission with these vaccines.

7.2 Desirable characteristics for vaccine manufacture and stockpiling

7.2.1 Safety for manufacturing under BL2 conditions in the U.S.

The SynCon® FMD vaccines are considered BSL 1 material (personal communication from personnel at VFX Animal Health), and thus, exceed this requirement.

7.2.2 Vaccine organism is not a select agent

Plasmids are not considered to be select agents.

7.2.3 Capability of meeting 9 CFR regulatory requirements for purity, potency, safety, and efficacy

The SynCon® FMD vaccines are still in the early stages of development. A few DNA vaccines, including two for prophylactic use, have become commercially available in the last few years, demonstrating that some DNA vaccines have the ability to meet 9 CFR regulatory requirements. The two prophylactic vaccines are a West Nile vaccine for horses, approved for use in the U.S. (West Nile Innovator®, Fort Dodge), and a hematopoietic necrosis vaccine for salmon, approved in Canada (Apex®-IHN, Novartis).[196,202] In addition, a therapeutic vaccine for canine melanoma (ONCEPT® Canine Melanoma Vaccine, Merial) was approved for use in dogs in the U.S.[196,202]) and a DNA construct expressing growth hormone releasing hormone (LifeTide® SW 5) has been licensed for use in pigs in Australia and New Zealand.[209]

7.2.4 Capability of meeting requirements for cost effective manufacturing and all proprietary rights to vaccine antigen, vectors, and/or adjuvants

DNA vaccines are potentially less expensive than other veterinary vaccines, as they can be produced in large quantities by bacteria, and high biosafety level facilities are not needed.[198,202] The manufacturing process for the SynCon® family of vaccines is well established and viable at commercial scale; the same process is already in use for LifeTide® SW 5, which is also manufactured by Inovio Pharmaceuticals, and is

commercially available in Australia and New Zealand (personal communication from personnel at Inovio Pharmaceuticals). There have been no manufacturing scale up issues identified.

7.2.5 Desirable characteristics for stockpiling for emergency use

7.2.5.1 Ongoing manufacture and sale in endemic countries which enables indefinite delivery/indefinite quantity (IDIQ) contracts for just in time delivery

Currently, there are no contracts to produce FMD DNA vaccines in endemic countries. Several FMD serotype-specific vaccine candidates are in the research stage in the U.S. (personal communication from personnel at DHS)

7.2.5.2 Stability when stored as bulk constructs

When stored at –80°C, research plasmids have an almost indefinite shelf life and have been known to remain active as long as 10-15 years after manufacture (personal communication from personnel at VGX Animal Health).

7.2.5.3 Long shelf-life and stability of finished vaccine

DNA vaccines are biologically stable and also have good thermostability.[196,198,202,203] The manufacturer anticipates that the shelf life of the SynCon® FMD vaccines will mirror that seen with LifeTide® SW 5, which is manufactured using the same process (personal communication from personnel at VGX Animal Health). A 4-year dating has been granted for the latter product in Australia (personal communication from personnel at VGX Animal Health). The product literature for LifeTide® SW 5 indicates that it should be stored frozen (-18°C); however, thawed, unusual vials may be stored for up to 6 months at refrigeration temperatures (2-8°C).[210]

7.2.5.4 Rapid scale up and manufacture in the US in an emergency

If there are facilities and trained personnel ready to respond, manufacturing scale up should not be an issue with plasmid vaccines (personal communication from personnel at Inovio Pharmaceuticals). The manufacturing process that would be used for the SynCon® FMD vaccines is already in use for the commercial LifeTide® SW 5 product. The SynCon® vaccines could be made in existing or dedicated plasmid production facilities,

using standard manufacturing techniques in commercial use. In theory, any facility capable of aerobic bacterial culture could also be converted to plasmid manufacturing (personal communication from personnel at VGX Animal Health).

In an emergency situation, the entire manufacturing process and release testing could be completed in 7-14 days per vaccine serial (personal communication from personnel at VGX Animal Health), once the gene has been sequenced and the manufacturing master seed is available (see section 7.2.6). Serial size would only be limited by the standing bacterial fermentation "warm base" deemed necessary by DHS/USDA. If stocks were held in bulk, field ready product could be deployed within 7 days of an outbreak.

7.2.6 Rapid conversion of manufacturing to outbreak strain

A single vaccine that is protective against multiple serotypes [208] and broadly cross-reactive within a serotype might reduce the need for conversion to an outbreak strain.

If a new FMD vaccine were required, the manufacturer estimates that a new master seed could be produced within a 2-3 week time frame, if one was not designed in advance (personal communication from personnel at VGX Animal Health). Full evaluation of each construct for U.S regulatory approval would be expected to take 3-5 years[2] unless a conditional license is issued. However, vaccines expressing new FMDV antigens might become available sooner, if the SynCon® FMD vaccines qualify for inclusion as a production platform under APHIS guidelines for licensing production platforms (Veterinary Services Memorandum 800.213).

7.3 Desirable characteristics for vaccine administration

7.3.1 Cost effective and practical delivery methods

DNA vaccines have good thermostability, which results in decreased dependence on a reliable cold chain during vaccination campaigns.[196,198,202,203] A vaccine that will be injected into a single site would clearly be simpler to administer than one that must be injected into multiple sites.

One of the challenges for DNA vaccination is that only a small proportion of the injected DNA enters a cell and reaches the nucleus.[169,200-202] Although unprotected DNA can be injected by needle, 95-99% remains outside cells and is degraded within 90 minutes.[200] Formulating DNA vaccines in microparticles or liposomes can increase cellular uptake of the plasmid.[197,202] Particle bombardment ("gene gun"), high-pressure delivery and

electroporation also improve uptake.[196-198,202] The method of delivery can influence the type, as well as the level, of the immune response.[16,64,196,200,203]

In the initial experiments, the SynCon® universal FMD vaccine was administered by electroporation.[208] Electroporation involves the application of electrical stimulation to muscles, to increase the efficiency of plasmid uptake by cells.[197,198,201] It can be used with both intradermal and intramuscular administration.[202] The feasibility of electroporation for field use in animals will depend on a cost benefit analysis, balancing the benefits of better potency with the cost of the devices and the added complexity of the vaccination process.[198,207] Improvements such as the development of portable battery operated devices might simplify their use.[207] The severe discomfort caused by the procedure[198] should also be considered. Some newer devices, or novel methods, such as noninvasive electroporation combined with intradermal injection of plasmid DNA, might cause less pain.[198,202]

7.3.2 Safety for humans accidentally exposed to the vaccine

Plasmid vaccines cannot multiply or reproduce themselves in mammalian cells. After accidental injection without electroporation, only very small amounts of DNA would be expected to enter cells. Most of the injected DNA remains at the injection site.[200] The highest concentrations are found during the first several minutes, with only trace amounts detected after several hours.[200] Small amounts of DNA are also distributed to other vascularized organs, but do not persist long.[200] Plasmids that reach the nucleus of cells might be expressed for a time.[200] This period can vary from several weeks to a few months, and sometimes longer (e.g., more than 2 years in mice in one study).[200] Although the expression of an FMDV antigen seems likely to be innocuous, two general safety concerns with DNA vaccination should be addressed. Both issues have been topics of discussion mainly in the context of DNA vaccines developed for humans and injected deliberately; however, the same risks could apply in case of accidental injection.

One theoretical concern has been the possibility that a plasmid might integrate into the host genome, and activate proto-oncogenes, inactivate tumor suppressor genes or cause chromosomal instability, potentially increasing the risk of tumor formation or causing other adverse effects.[203] Only a few studies have investigated the integration of injected plasmids into chromosomal DNA, with most concluding that there was a negligible risk.[197,198,200] Most of these studies were unable to distinguish residual plasmids in the nucleus from integrated plasmids; however, one group used a technique that detected a small number of recombination events in mice, following

electroporation.[200] The rate of these events was less than the rate of spontaneous mutation in the genome. Some authors note that plasmids with new backbones for increased gene expression might be more likely to integrate.[198]

A second concern in people has been the possibility that the foreign DNA might induce anti-nuclear antibodies and cause or exacerbate autoimmune conditions.[203] Animal studies to date suggest that, although anti-DNA autoantibodies may increase, these vaccines do not elevate the risk of autoimmunity.[197,198] However, immune complexes might cause pathology after prolonged expression of antigens.[211 cited in 198]

Currently, a number of human clinical trials are investigating DNA vaccines, most either as therapeutic vaccines for cancer or prophylactic vaccines for HIV.[197] Vaccines for a few other viral pathogens are also in clinical trials.[197] The safety record, to date, has been good, and the most common side effect has been mild to moderate inflammation at the injection site.[198]

7.4 Desirable characteristics for minimizing the impact on food production and animal trade

7.4.1 Safety for use in food producing animals with no, or reasonably short, withdrawal time for animal products for human consumption

7.4.1.1 Absence of virulent viruses and extraneous pathogens

DNA vaccines are noninfectious and cannot revert to a virulent form.[196,198] As with other types of vaccines, good quality control for plasmid identity, purity and sterility is important.[199] An advantage to DNA vaccines is that there is little risk they would become contaminated with pathogens of animal origin during production, as they are produced in bacteria.[196]

7.4.1.2 Allergic reactions or other adverse effects in vaccinated animals

Preliminary results in swine demonstrated no local or systemic reactions at the doses used for SynCon® FMD vaccines (personal communication from personnel at DHS)

7.4.1.3 Withdrawal period and safety for human consumption

The withdrawal period for SynCon® FMD vaccines has not been established. However, VGX Animal Health anticipates that the safety profile of these vaccines will mirror that

of LifeTide® SW 5 (a GHRH-expressing plasmid), which is produced by the same company (personal communication from personnel at VGX Animal Health). The latter product has a zero day slaughter withholding period in meat from swine, when it is used according to label directions.[210] This product is not currently licensed in the U.S., but it is approved for use in pigs in Australia and New Zealand. It should be noted that all vaccines for food animals in the U.S. must be labeled with a minimum slaughter withdrawal time of 21 days.

A DNA vaccine has been approved for use in fish in Canada, and is considered safe for consumers who eat the fish.[202] The manufacturer of the SynCon® FMD vaccines also states that there is no known issue that would cause food safety concerns with these vaccines (personal communication from personnel at VGX Animal Health).

7.4.2 Availability of companion diagnostic test to detect infections in vaccinated animals (DIVA)

Theoretically, a number of NSP serological tests could be used with SynCon® FMD vaccines encoding capsular antigens.[208] DIVA capability would be expected to allow DNA vaccines to be used with surveillance programs intended to document freedom from infection for international trade. However, this will require validation of the DIVA assays for this purpose.

7.5 Desirable characteristics for controlling FMD in wild and feral populations

7.5.1 Safety and efficacy when delivered orally in baits for feral swine or deer

The temperature stability of DNA vaccines might make them attractive candidates for oral vaccines in wildlife.[202] Oral DNA vaccines have been investigated in diverse species including fish,[212] pigs[213] and mice.[214] Various approaches such as DNA incorporation on PLG or chitosan particles have been explored.[212,214,215] There are no published studies investigating oral DNA vaccines for FMD.

Section II: Antiviral Prophylaxis for the Control of Foot and Mouth Disease

Researchers have investigated a number of approaches to inhibit FMDV during the initial period after vaccination, when the immune response has not yet developed and animals are most vulnerable to infection. Antiviral prophylaxis could also be used to protect unusually valuable animals, such as exotic species in zoos or valuable breeding stock, as well as young animals that still have maternal antibodies and cannot be effectively vaccinated.[216] In addition, it might be used as a control measure to reduce transmission from animals that have been exposed to FMDV.[217] Potential approaches to antiviral prophylaxis are diverse, and include cytokines, cytokine inducers and non-specific stimulators of innate immunity (e.g., CpG), as well as antiviral drugs and nucleic acid constructs that can inhibit FMDV replication. Some of the factors that should be considered in evaluating antiviral approaches include their efficacy, safety/ side effects and the potential for drug-resistance.[216] The onset and duration of the antiviral effects, in addition to the ability to suppress virus replication, are important considerations in evaluating effectiveness. Cost can also be a significant factor, particularly given the large body mass of most livestock.[216] Administration and storage needs must be considered in large-scale applications.[216] Lastly, safety specifically for use in food producing animals is important when treated animals will enter the food chain.

At present, most antiviral agents have only been examined in cells or laboratory rodents; relatively few have been tested in livestock. Most of the published experiments in livestock have evaluated interferons or interferon inducers, particularly human adenovirus 5 (hAd5) vectored interferon constructs. A limited number of papers have investigated other approaches, such as RNA interference or antiviral drugs. To date, all animal testing has been conducted under controlled laboratory conditions, using healthy animals.[216] It will be important to supplement these studies with field tests, to determine potency in animals that may be in less than optimal health or infected with other microorganisms.[216]

1. Antiviral drugs

Antiviral drugs have been evaluated mainly in FMDV-infected cell cultures, and in some cases, in laboratory rodent models. A number of drugs including ribavirin, 5-fluorouracil, 5-azacytidine, 2'-C-methylcytidine, 6-azauridine, guanidine-HCl, chitosan, T-705 (favipiravir), and the thiol protease inhibitors L-trans-epoxysuccinyl-leucylamido(4-

guanidino)butane (E-64) and its membrane-permeable analog E-64d, were promising in these systems.[22,216,218-220] The effectiveness of most drugs in FMDV-infected livestock is currently unknown. However, the pyrazinecarboxamide derivate T-1105 demonstrated efficacy in pigs, as well as in cell cultures.[221] In a presentation that appears to be unpublished, pigs that were given 200 mg/kg T-1105 twice daily in feed, beginning 1 hour before FMDV challenge and continuing for 6 days, were protected from clinical signs. Viremia was not detected by PCR, and FMDV was not isolated from nasal swabs. Antibody titers to FMDV were also very low, suggesting that virus replication was limited. One potential advantage to this drug is the method of administration.

Tissue residues can limit the use of antiviral drugs against FMDV, as some drugs are not considered acceptable in animals that will enter the food chain. Drugs that cannot be employed in food-producing animals might still be used to prevent disease spread, with subsequent disposal of the animals. In addition, they might be acceptable in some FMDV-susceptible species not used for food, such as animals in zoos. Cost can also be a limiting factor in the use of antiviral drugs.

2. Cytokines and related approaches

Among the cytokines, only the interferons have been investigated extensively for their ability to inhibit FMDV. Interferons have been shown to suppress FMDV replication in cell cultures,[21,22] and genetic constructs that express interferons have been evaluated in a number of studies in livestock. One advantage to interferons, compared to antiviral drugs, is that viruses do not become resistant to these agents.[222] Another is that they occur naturally in the body, making them a good choice for use in food producing animals.

At present, three types of interferons (types I, II and III) are known. The best characterized members of the type I interferon group are IFNα, which has several isoforms, and IFNβ.[222] Type I interferons play an important role in inducing antiviral responses in infected cells, and are also involved in the production of innate immune responses.[223] Although type I interferons all act through one receptor, the effects of different agents or isoforms are not necessarily identical.[224] For example, IFNα has been shown to be more effective than IFNβ for at least one therapeutic use in humans.[222] Such variations are thought to be due, at least in part, to differences in their interactions with the type I IFN receptor.[224]

Type II interferon (IFNγ) is important in activating components of cell-mediated immunity (CMI) and inducing the differentiation of Th1 cells, although it also has

antiviral activity.[222,223] Type III interferons (IFNλ), which were discovered in 2003, are less well characterized. Type III interferons share intracellular signaling pathways with type I interferons, and induce antiviral responses; however, they function through different receptors, and are more closely related to the IL-10 family of cytokines.[222] The three type III interferons are also known as IL-29 (IFNλ1), IL-28A (IFNλ2) and IL-28B ((IFNλ3).[222] While IFN α/β receptors and IFNγ receptors are widely expressed, only a limited number of cell types express the receptor for IFNλ.[222] Importantly, they include epithelial cells in the skin and mucosa, which are targets of FMDV.[225-229 cited in 230]

Therapeutic uses of interferons have been characterized more extensively in people than livestock, as some agents are licensed for human use in chronic viral infections or cancer.[222] Type I interferon (mainly human IFNα) is the most commonly used IFN in people, but human IFNγ (type II) is employed to a limited extent, and human IFNλ (type III IFN) is in at least one clinical trial.[222] In people, interferons that are conjugated with polyethylene glycol or albumin are reported to have improved pharmacokinetics, with more stable drug concentrations and prolonged activity.[222] However, there have been safety concerns with certain agents, such as one formulation of IFNα conjugated with albumin, which was withdrawn from the market.[222] The most common side effects of interferons in humans include transient influenza-like signs, myelosuppression (including neutropenia and thrombocytopenia), rashes and mild injection site reactions, neuropsychiatric manifestations, and the development or exacerbation of autoimmune conditions.[222,224] Rare side effects, such as adverse effects on pulmonary function, have been reported as these drugs become more widely used.[222] Some of these side effects, particularly transient fever, have also been reported from studies in animals. Pulmonary hypertension was demonstrated in sheep treated with IFNα,[222] and myelosuppression has been seen in mice.[231]

2.1 Conjugated interferons to suppress FMDV

Currently, there are no conjugated species-specific interferons licensed for use in livestock. Unmodified interferons have a short half-life in the blood.[232,233 cited in 234] Together with the dose-dependent side effects, this can limit their usefulness as prophylactic agents in animals.[232,233 cited in 234] One recent study examined the use of recombinant porcine IFNγ fused with glutathione S-transferase, for the prevention of FMD in pigs.[235] Pigs treated with this agent and challenged 2 days later were completely protected from clinical signs at the highest dose (30 mg/animal), and partially protected by lower doses (10-20 mg/animal).[235] Transient fever was reported for 2-4 hours after injection of the conjugated interferon.

Human adenovirus 5 (hAd5)-vectored constructs that express various interferons have been examined extensively in swine challenged with FMDV. Expression of interferon from such constructs can prolong its effects, and adverse effects can be decreased by administering smaller amounts of the construct.[234]

In the initial studies, hAd5-vectored porcine IFNα (10^9 pfu/ animal) protected 3 pigs challenged one day later with FMDV A_{24} Cruzeiro.[236] These pigs did not develop clinical signs or detectable viremia. In addition, the absence of measurable antibody titers to NSPs suggested that there was little or no virus replication. Antiviral activity was detected as soon as 16 hours after the administration of hAd5-vectored IFNα, and lasted for up to 5 days. A lower dose of the construct (10^8 pfu/ animal) resulted in lower levels of antiviral activity, which persisted for up to 3 days and partially protected pigs from clinical signs upon challenge.[236] Most of the IFN-treated pigs developed elevated temperatures (which reached 40ºC for 2-3 days), but other adverse effects were not seen.[236] One of the 3 pigs given the highest dose of the construct died of causes unrelated to FMD, 12 days later.[236] Massive peritonitis, most likely caused by perforation of the ileum, was found at necropsy.

A follow-up study examined the onset and duration of protection, using 10^9 pfu/ animal of hAd5-vectored IFNα.[217] One potential complication in this experiment is that all of the pigs had a skin rash, unrelated to the experiment, which the authors felt may have compromised the animals' health. As reported in the previous study, antiviral activity was highest on the day after the pigs were inoculated, and decreased the following day; however, some activity was still detectable for the next 3-4 days. A single injection of hAd5-vectored IFNα provided complete clinical protection from FMDV for at least 3 days, and possibly longer. Six treated pigs that were challenged after 1-3 days with FMDV A_{24} Cruzeiro remained asymptomatic, while 3 pigs challenged after 5 days were either partially or completely protected from clinical signs. Another group of 3 animals challenged after 5 days, in a later, unrelated experiment, all remained asymptomatic and had no detectable viremia. For this reason, the authors suggested that the weaker protection at 5 days in the first trial may have been caused by the animals' compromised health. However, individual animal variability might also account for the difference in the construct's effectiveness in the two trials. Only limited efficacy was seen in pigs challenged after 7 days. These animals had lower levels of virus in the blood compared to the controls, and the clinical signs developed later. In this study, hAd5-vectored IFNα also protected pigs partially or completely from clinical signs when it was administered one day after exposure to FMDV. As in the previous experiment, the only

reported side effect was elevated body temperature, lasting 1-2 days, in some animals. Two deaths occurred in pigs that were partially or completely protected from clinical signs of FMDV. Both deaths were attributed to bacteremia affecting the brain. One of these pigs died 11 days after challenge (12 days after administration of the IFN construct), and had abscesses in the lung, thyroid and brain stem. The other animal died 8 days after challenge (15 days after administration of the IFN construct), most likely from meningitis after dissemination of bacteria from skin abscesses.

A later study examined the effects of hAd5-vectored porcine IFNγ, alone or combined with hAd5-vectored porcine IFNα. Pigs that received either a low dose of hAd5-IFNα (10^8 pfu/ animal) or a low dose of hAd5 IFNγ (10^8 pfu/ animal) were partially protected from clinical signs when they were challenged one day later with FMDV A_{24} Cruzeiro.[237] Pigs that received either a high dose (10^{10} pfu/ animal) of hAd5 IFNγ alone, or low doses of both constructs, did not develop clinical signs or detectable viremia, and FMDV was not found in nasal swabs.[237] Although these results are useful in assessing the relative effects of higher and lower doses of IFNα and IFNγ, it should be noted that the amount of interferon expressed from the hAd5 constructs may vary between experiments. In a later experiment, only partial protection was seen in pigs inoculated with 10^9 pfu/animal of hAd5-IFN-α, 10^{10} pfu of hAd5-IFN-γ, or both constructs.[223] Plasma IFN-α levels in the latter study were low, compared to previous studies, and plasma IFN-γ levels were undetectable. Possible explanations for the discrepancy between the two experiments include the use of slightly different promoters in the constructs, the use of higher passage vectors in the second experiment (which may have included some constructs that did not contain interferon genes), and the administration of a higher challenge dose.[223]

Further studies demonstrated that, when animals were challenged one day after inoculation, hAd5-vectored porcine IFNα was effective not only against FMDV A_{24} Cruzeiro (which was used in all earlier experiments), but also against FMDV O_1 Manisa and Asia-1.[238] Protection against direct contact challenge with A_{24} Cruzeiro-infected pigs was also shown.[238]. Dose-response studies in this series of experiments found that 10^{11} FFU/animal was required to eliminate clinical signs, detectable viremia and virus isolation from nasal secretions; 10^{10} FFU/ animal, administered at a single site, resulted in only partial protection against any FMDV serotype. However, administration of the higher dose (10^{11} FFU/animal) of either hAd5-porcine IFNα or hAd5-porcine IFNβ caused jaundice and loss of appetite, and animals took 2-3 days to recover.[238] Administration of hAd5-IFNα intramuscularly at 4 sites in the neck, rather than as a single dose in the leg, allowed a 10-fold reduction in dose, with equivalent protection against clinical signs, viremia and virus shedding in 2 pigs.[238] Injection of hAd5-IFNα into 4 sites in the leg was

less effective. One of the 3 pigs inoculated at 4 sites in the neck died before challenge, and one of 3 pigs inoculated at 4 sites in the hind legs died one day after challenge. In both cases, the causes were unspecified, but stated to be unrelated to administration of the vector or FMDV, based on histopathology at postmortem.

One study examined the co-administration of hAd5-vectored porcine IFNα and an hAd5-vectored A$_{24}$ Cruzeiro vaccine, with challenge after 5 days.[217] Pigs that received either hAd5-IFNα alone, or both constructs, were protected from clinical signs, fever, viremia and virus shedding, with no evidence of virus replication. Pigs that received both constructs also had higher levels of neutralizing antibodies to structural FMDV proteins after challenge, compared to vaccine alone. This suggests that the IFNα may have acted as a vaccine adjuvant, as well as protecting the animals directly during the early period after vaccination. Pigs that only received the vaccine were partially protected from clinical signs, and shed FMDV at similar levels as unvaccinated pigs.[217]

2.3 Human adenovirus-vectored interferon constructs in cattle

Three published experiments investigated the use of hAd5-vectored type I or type III interferon constructs in cattle. To date, only partial protection has been demonstrated in this species. In the first study, hAd5-vectored bovine IFNα (10^{10} pfu/animal) resulted in milder and delayed clinical signs, with a slower onset of viremia and virus shedding, when the animals were challenged 1-2 days later with FMDV A$_{24}$ Cruzeiro.[234] Fever, seen during the first 1-2 days, was the only side effect of IFN treatment. Although interferon activity was detected for 2-4 days, this construct appeared to produce lower levels of biologically active interferon than the hAd5-vectored porcine IFNα construct used in pigs.[234]

Later studies tested the effects of bovine IFNλ3 (type III), based on the premise that this cytokine may be involved in inducing an antiviral state in the skin and upper respiratory tract, where FMDV replicates.[239] In the initial experiment, hAd5-vectored IFNλ3 was shown to induce systemic antiviral activity and up-regulate the expression of interferon-response genes in cattle.[239] A subsequent study tested whether hAd5-IFNλ3 (10^{11} PFU/animal), alone or combined with hAd5-vectored porcine IFNα (10^{11} PFU/animal), could protect cattle challenged one day later with serotype A$_{24}$ Cruzeiro viruses (by intradermolingual inoculation) or serotype O$_1$ Manisa viruses (in aerosols).[230] The porcine IFNα construct was used in this experiment, rather the bovine IFNα construct, as it had slightly higher interferon activity. Administration of hAd5-IFNλ3, alone or combined with hAd5-porcine IFNα, resulted in delayed and milder clinical signs in challenged cattle, with reduced viremia and virus shedding. A lower dose of both

constructs was less effective, while the IFNα construct alone seemed to have little or no effect.

2.4 Interferon inducers: oligonucleotides and chemical agents

Oligonucleotides and chemicals that induce interferons have been examined alone, and in combination with hAd5-vectored interferon, for their effects on FMDV. Most studies have tested these agents in cells and/or mice. Some promising agents have also been evaluated in livestock, with mixed results.

Unmethylated CpG is a nonspecific antiviral agent that induces IFN synthesis and cellular activation.[216] In mice, oligonucleotides containing unmethylated CpG motifs decreased the severity of clinical signs and viremia, when the animals were challenged 4 days later with FMDV.[240 cited in 216] Antiviral effects were seen even when the mice were given the drug 12 hours after challenge. However, an unpublished study suggests that CpG cannot protect livestock from FMDV. (Alves et al., manuscript in preparation) [cited in 241] Other agents reported to protect mice but not pigs from FMDV challenge include itaconic-acrylic acid copolymer (IAA or HMW),[242] divinyl ether-maleic anhydride copolymer (pyran),[242] and the synthetic interferon inducers polyacrylic acid and chlorite-oxidized amylose (COAM).[243 cited in 242] Some of these agents also had significant adverse effects in swine, when they were administered by some routes. Intraperitoneal administration of DVE/MA caused peritonitis and death at high doses, and asymptomatic chronic fibrinous peritonitis at a lower dose.[242] In contrast, IAA was not toxic when administered by the same route.[242]

Polyinosinic:polycytidylic acid (poly IC) and polyriboinosinic-polyribocytidylic acid stabilized with poly-l-lysine and carboxymethyl cellulose (poly ICLC) have also been tested against FMDV. Poly IC and poly ICLC are synthetic, double-stranded RNA molecules that induce interferons by mimicking viral nucleic acids.[244-246 cited in247] Poly IC was effective against FMDV challenge in mice,[248 cited in 241] but not in two livestock studies.[242;249 cited in 241] In cattle, poly IC had no effect on the severity of clinical signs in animals challenged with FMDV (serotype O) 2-6 hours later, although interferon was detectable one hour after the agent was administered.[242] Similarly, poly IC was ineffective in goats challenged with FMDV after 24 hours,[242] or in pigs.[249 cited in 241] Poly IC also caused significant adverse effects in goats. While an elevated body temperature, lasting 5-6 hours, was the only side effect in cattle, mild to severe clinical signs occurred in most goats that received a higher dose of poly IC, and some goats that received a lower dose.[242] Some affected goats developed elevated temperatures, with urination, signs of apparent anxiety, and rapid and shallow breathing, which persisted for 15

minutes to 2 hours. Some of these animals remained lethargic for up to 24 hours. A few goats had foul-smelling diarrhea for 1-2 days. One goat was found dead 24 hours after the administration of poly IC, with disseminated petechial hemorrhages and blood in the lumen of the intestines, and degenerative lesions of the kidney and liver. Similar side effects, such as transient depression, increased respiratory rates and liver degeneration, have also been reported in calves and dogs.[250,251 cited in 242]

Poly ICLC has better biostability in animals than poly IC,[252 cited in 247] and a recent experiment evaluated whether this agent could be given with hAd5-vectored IFN, to decrease the dose of the interferon construct and improve its cost-effectiveness.[247] In this study, 2.5 x 10^9 FFU hAd5-porcine IFNα, given alone, decreased but did not eliminate clinical signs, viremia and virus shedding in pigs challenged with FMDV one day later. A combination of poly ICLC (8 mg/animal) and a lower dose of hdAd5 IFNα (1 x 10^9 FFU/animal) prevented clinical signs in 3 animals, with no evidence of virus replication by RT-PCR and no antibodies to NSPs. The same dose of poly ICLC (8 mg/animal), without hdAd5 IFNα, also provided complete protection when it was administered to 2 pigs. However, 8 mg of poly ICLC and 2.5 x 10^8 FFU hdAd5-IFNα was only partially protective in 3 other animals. The authors postulated that individual animal variability might account for this discrepancy. A lower dose of poly ICLC (4 mg/ animal), alone or combined with hdAd5 porcine IFNα, was less effective. No inoculation site reactions or loss of appetite were reported after inoculation of poly ICLC.[247] Two pigs died in this experiment, both of unspecified causes that were stated to be unrelated to FMDV. One of these animals received 8 mg poly ICLC and died one day after FMDV challenge, while the other was inoculated with 4mg poly IC and 1x10^9 FFU of hAd5-porcine IFNα, and died 7 days after FMDV challenge.

Other combinations of hAd5 IFNα and antivirals might also be effective, but have not yet been tested in livestock. The combination of ribavirin and hAd5-vectored IFNα was protective against FMDV in both cultured cells and mice.[22]

2.5 Interferon inducers: alphavirus replicon vectors

One recent study investigated the use of an alphavirus replicon vector based on Venezuelan equine encephalitis (VEE) virus. Treatment of FMDV-infected cells with either VEE empty replicon particles or porcine IFNα cloned into the VEE replicon vector blocked FMDV replication in cells.[253] Empty VEE replicon particles were also tested in mice, which were protected from FMDV challenge.[253] Empty VEE replicon particles are reported to upregulate inflammatory cytokines and chemokines, and appear to transiently induce an inflammatory environment in the draining lymph node.[177] In the

mice, protection was attributed to the induction of IFNα but not IFNβ, IFNγ or IFNλ. Porcine IFNα cloned into the VEE replicon vector was not tested in mice, as this form of interferon was not thought to be effective in this species.

3. Nucleic acid strategies, including RNA interference

Nucleic acid strategies have also been tested for their ability to suppress FMDV replication. Some methods that appear promising in cells and laboratory rodent models include the use of small interfering RNAs (siRNA),[216,254-259] micro RNA[260] and *in vitro* transcribed RNAs that mimic structural domains in noncoding regions of FMDV and induce cytokines and innate immune responses.[261] Currently, only the siRNA strategy has been tested in any livestock species.[254,255]

Small interfering RNAs are short, double-stranded RNA molecules that are homologous to part of the target gene, and cause their complementary mRNA in the cell to be degraded.[216,254,255] This posttranscriptional gene silencing mechanism, called RNA interference, is thought to be an evolutionarily conserved mechanism in eukaryotes.[255] It may function in gene regulation and in helping to maintain the stability of the genome, and it is also thought to be a viral defense mechanism in plants and insects.[254];[262 cited in 255] Because of the rapidity and specificity of RNA interference, siRNA has been investigated as one method of inhibiting pathogen replication, either alone or combined with other agents. One impediment to using siRNA is that, for optimal efficacy, the vector used to express the RNA should have a similar distribution in tissues as FMDV.[254,255] Other challenges include the short duration of the siRNA effect, and its inability to completely clear viruses.[254] In addition, there are still uncertainties in how well this system would work in the field.[216]

Two studies examined the siRNA system in FMDV-infected livestock, and also in cells and laboratory rodents. In one experiment, hAd5-vectors that expressed short-hairpin RNAs against either the FMDV structural protein 1D (hAd5-NT21) or the 3D polymerase (hAd5-POL) were initially shown to suppress the replication of homologous FMDV in swine cells.[254] The hAd5-POL construct also inhibited the replication of heterologous FMDV in cells, and it was partially protective against homologous challenge, 1 day after administration, in a guinea pig model.[254] The siRNA technique was then tested in swine. A mixture of hAd5-NT21 and hAd5-POL protected 2 of 3 pigs from clinical signs, and resulted in delayed and milder clinical signs in the third animal.[254] A lower dose resulted in full clinical protection in one animal and partial protection in two pigs. Viremia also appeared to be decreased in treated pigs, although it was not systematically examined.

One difficulty identified in this experiment is that the hAD5-vectored constructs do not seem to concentrate in the tissues where FMDV usually replicates.[254] In guinea pigs, FMDV occurred mainly in the epithelial cells of clinically affected feet, but the vast majority of the hAd5 vector was found in the liver. An additional concern with the use of hAd5 vectors is that the VA1 noncoding region of adenoviruses can inhibit the biogenesis of siRNA and microRNA.[263 cited in 254]

A later study employed a different vector, and also focused solely on RNA interference directed against conserved regions of the FMDV genome. This may be a safer strategy than targeting siRNA to structural proteins, as the latter agents may not be well matched to the virus in an emerging outbreak.[264-267 cited in 255] In this experiment, plasmids that expressed siRNA were generated against highly conserved sequences within the NSPs 3D and 2B.[255] These plasmids decreased the replication of serotype O and Asia1 viruses in cells, and were promising in mice. An attenuated *Salmonella choleraesuis* (C500) vaccine strain that expressed the siRNA against 3D was then constructed. This vector was chosen because attenuated *S. choleraesuis* tends to localize to the lymph node, tonsil, lung and gastrointestinal tract of pigs.[268 cited in 255] In a laboratory rodent model, *S. choleraesuis*-vectored 3D siRNA protected 80% of guinea pigs challenged after 36 hours.[255] Lastly, swine given 5×10^9 CFU of this construct and challenged one day later with serotype O FMDV (strain HKN/2002) had milder and delayed clinical signs compared to controls. Antibody titers to NSPs were also much lower, suggesting that virus replication was decreased. A higher dose of the construct was less effective, possibly because low doses of the *Salmonella* vector are better able to evade anti-vector immune responses.[255]

4. Summary

Currently, hAd5-vectored IFN is the only form of antiviral prophylaxis that has been evaluated extensively in livestock. While this technique is promising in swine, the need for high doses of these constructs could be a challenge for widespread use in large numbers of animals.[238,247] Injection into multiple sites results in a tenfold reduction in the amount needed,[238] but this method will also complicate administration in the field. In addition, the dose needed to suppress FMDV replication is still relatively high, and the technique thus remains costly.[247] Co-administration with other antivirals may allow additional dose reductions.[22,247] The ability of hAd5-vectored IFN to protect cattle from FMDV is still in question, as constructs expressing type I or type III interferons provided only partial protection in two studies.[234,239] These agents have not been tested in small ruminants in the U.S., and there are no published reports of testing in other countries.

There is still limited information on other forms of prophylaxis. One antiviral drug, T-1105, appeared to be promising against FMDV challenge in pigs;[221] however, these experiments have apparently not been formally published. In addition, this agent does not seem to have been tested yet in other livestock species. The interferon inducer poly ICLC also seemed to be effective in some animals, when used alone.[247] RNA interference may provide some protection; however, siRNA has only been tested in pigs, which were partially protected in the two published experiments.[254,255] Nevertheless, it is intriguing to note a recent report that hAd5-vectored IFNα combined with hAd5-vectored siRNA was synergistic in cultured cells and mice.[22] Antiviral agents will need to be tested in each animal species that will be treated, as potential adverse effects, as well as effectiveness, may vary between species.

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