NAHEMS GUIDELINES:
VACCINATION FOR CONTAGIOUS DISEASES
APPENDIX A: FOOT-AND-MOUTH DISEASE

FAD PReP
Foreign Animal Disease
Preparedness & Response Plan

USDA

NAHEMS
National Animal Health
Emergency Management System

the Center for Food Security & Public Health
Iowa State University®

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The Foreign Animal Disease Preparedness and Response Plan (FAD PReP)/National Animal Health Emergency Management System (NAHEMS) Guidelines provide a framework for use in dealing with an animal health emergency in the United States.

This FAD PReP/NAHEMS Guidelines was produced by the Center for Food Security and Public Health, Iowa State University of Science and Technology, College of Veterinary Medicine, in collaboration with the U.S. Department of Agriculture Animal and Plant Health Inspection Service through a cooperative agreement.

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THE IMPERATIVE FOR FOREIGN ANIMAL DISEASE PREPAREDNESS AND RESPONSE

Why Foreign Animal Diseases Matter

Preparing for and responding to foreign animal diseases (FADs)—such as highly pathogenic avian influenza (HPAI) and foot-and-mouth disease (FMD)—are critical actions to safeguard the nation's animal health, food system, public health, environment, and economy. FAD PReP, or the *Foreign Animal Disease Preparedness and Response Plan*, prepares for such events.

Studies have estimated a likely national welfare loss between $2.3–69 billion[1] for an FMD outbreak in California, depending on delay in diagnosing the disease.[2] The economic impact would result from lost international trade and disrupted interstate trade, as well as from costs directly associated with the eradication effort, such as depopulation, indemnity, carcass disposal, and cleaning and disinfection. In addition, there would be direct and indirect costs related to foregone production, unemployment, and losses in related businesses. The social and psychological impact on owners and growers would be severe. Zoonotic diseases, such as HPAI and Nipah/Hendra may also pose a threat to public health.

Challenges of Responding to an FAD Event

Responding to an FAD event—large or small—may be complex and difficult, challenging all stakeholders involved. Response activities require significant prior preparation. There will be imminent and problematic disruptions to interstate commerce and international trade.

A response effort must have the capability to be rapidly scaled according to the incident. This may involve many resources, personnel, and countermeasures. Not all emergency responders may have the specific food and agriculture skills required in areas such as biosecurity, quarantine and movement control, epidemiological investigation, diagnostic testing, depopulation, disposal, and possibly emergency vaccination.

Establishing commonly accepted and understood response goals and guidelines, as accomplished by the FAD PReP materials, will help to broaden awareness of accepted objectives as well as potential problems.

[1] Carpenter TE, O'Brien JM, Hagerman AD, & McCarl BA. 2011. "Epidemic and economic impacts of delayed detection of foot-and-mouth disease: a case study of a simulated outbreak in California." *J Vet Diagn Invest.* 23:26-33.
[2] Estimates based on models may vary: Ekboir (1999) estimated a loss of between $8.5 and $13.5 billion for an FMD outbreak in California. Ekboir JM. 1999. "Potential Impact of Foot-and-Mouth Disease in California: the Role and Contribution of Animal Health Surveillance and Monitoring Services." *Agricultural Issues Center.* University of California, Davis.

Lessons Learned from Past FAD Outbreaks

The foundation of FAD PReP is lessons learned in managing past FAD incidents. FAD PReP is based on the following:

- Providing processes for emergency planning that respect local knowledge.
- Integrating State-Federal-Tribal-industry planning processes.
- Ensuring that there are clearly defined, obtainable, and unified goals for response.
- Having a Unified Command with a proper delegation of authority that is able to act with speed and certainty.
- Employing science- and risk-based management approaches to FAD response.
- Ensuring that all guidelines, strategies, and procedures are communicated effectively to responders and stakeholders.
- Identifying resources and trained personnel required for an effective incident response.
- Trying to resolve competing interests prior to an outbreak and addressing them quickly during an outbreak.
- Achieving rapid FAD detection and tracing.

FAD PReP Mission and Goals

The mission of FAD PReP is to raise awareness, expectations, and develop capabilities surrounding FAD preparedness and response. The goal of FAD PReP is to integrate, synchronize, and deconflict preparedness and response capabilities as much as possible before an outbreak by providing goals, guidelines, strategies, and procedures that are clear, comprehensive, easily readable, easily updated, and that comply with the National Incident Management System.

In the event of an FAD outbreak, the three key response goals are to: (*1) detect, control, and contain the FAD in animals as quickly as possible; (2) eradicate the FAD using strategies that seek to stabilize animal agriculture, the food supply, the economy, and to protect public health and the environment; and (3) provide science- and risk-based approaches and systems to facilitate continuity of business for non-infected animals and non-contaminated animal products.* Achieving these three goals will allow individual livestock facilities, States, Tribes, regions, and industries to resume normal production as quickly as possible. They will also allow the United States to regain FAD-free status without the response effort causing more disruption and damage than the disease outbreak itself.

FAD PReP Documents and Materials

FAD PReP is not just one, standalone FAD plan. Instead, it is a comprehensive U.S. preparedness and response strategy for FAD threats, both zoonotic and non-zoonotic. The following section provides examples of the different types of FAD PReP documents available.

- Strategic Plans—Concept of Operations
  - *APHIS Foreign Animal Disease Framework: Roles and Coordination* (FAD PReP Manual 1-0): This document provides an overall concept of operations for FAD preparedness and response for APHIS, explaining the framework of existing approaches, systems, and relationships.
  - *APHIS Foreign Animal Disease Framework: Response Strategies* (FAD PReP Manual 2-0): This document provides significant detail on response strategies that will be conducted in an FAD outbreak.
  - *Incident Coordination Group Plan* (FAD PReP Manual 3-0): This document explains how APHIS headquarters will organize in the event of an animal health emergency.
  - *FAD Investigation Manual* (FAD PReP Manual 4-0): This field-ready manual provides detailed information on completing an FAD investigation from start to finish.

  - *A Partial List of FAD Stakeholders* (FAD PReP Manual 5-0): This guide identifies key stakeholders with whom the National Preparedness and Incident Coordination (NPIC) Center collaborates.
- NAHEMS Guidelines
  - These documents describe many of the critical preparedness and response activities, and can be considered as a competent veterinary authority for responders, planners, and policy-makers.
- Industry Manuals
  - These manuals describe the complexity of industry to emergency planners and responders and provide industry a window into emergency response.
- Disease Response Plans
  - Response plans are intended to provide disease-specific information about response strategies. They offer guidance to all stakeholders on capabilities and critical activities that would be required to respond to an FAD outbreak.
- Standard Operating Procedures (SOPs) for Critical Activities
  - For planners and responders, these SOPs provide details for conducting critical activities such as disposal, depopulation, cleaning and disinfection, and biosecurity that are essential to effective preparedness and response to an FAD outbreak. These SOPs provide operational details that are not discussed in depth in strategy documents or disease-specific response plans.
- Continuity of Business Plans (commodity specific plans developed by public-private-academic partnerships)
  - Known as the Secure Food Supply Plans, these materials use science- and risk-based information to facilitate market continuity for specific products in an outbreak.
  - More information on these plans can be found at the following: www.secureeggsupply.com, www.securepork.org, www.securemilksupply.org, www.securebroilersupply.com.
- APHIS Emergency Management
  - APHIS Directives and Veterinary Services (VS) Guidance Documents provide important emergency management policy. These documents provide guidance on topics ranging from emergency mobilization, to FAD investigations, to protecting personnel from HPAI.

For those with access to the APHIS intranet, these documents are available on the internal APHIS FAD PReP website: http://inside.aphis.usda.gov/vs/em/fadprep.shtml. Most documents are available publicly, at http://www.aphis.usda.gov/fadprep.

PREFACE

The Foreign Animal Disease Preparedness and Response Plan (FAD PReP)/National Animal Health Emergency Response System (NAHEMS) Guidelines provide the foundation for a coordinated national, regional, state and local response in an emergency. As such, they are meant to complement non-Federal preparedness activities. These guidelines may be integrated into the preparedness plans of other Federal agencies, State and local agencies, Tribal Nations, United States Territories, and additional groups involved in animal health emergency management activities.

This Appendix A: Vaccination for Foot-and-Mouth Disease is a supplement to FAD PReP/NAHEMS Guidelines: Vaccination for Contagious Diseases, and covers the disease-specific strategies and general considerations of vaccination. Both documents are components of APHIS' FAD PReP/NAHEMS Guideline Series, and are designed for use by APHIS Veterinary Services (VS), and other official response personnel in the event of an animal health emergency, such as the natural occurrence or intentional introduction of a highly contagious foreign animal disease in the United States.

Appendix A: Vaccination for Foot-and-Mouth Disease, together with the Vaccination for Contagious Diseases Guidelines, provide guidance for USDA employees, including National Animal Health Emergency Response Corps (NAHERC) members, on emergency foot-and-mouth disease vaccination principles. The general principles discussed in this document are intended to serve as a basis for making sound decisions regarding vaccination in a foot-and-mouth disease emergency. As always, it is important to evaluate each situation and adjust procedures to the risks present in the situation.

The FAD PReP/NAHEMS Guidelines are designed for use as a preparedness resource rather than as a comprehensive response document. Additional resources are included in the References at the end of this document.

APHIS DOCUMENTS

Several key APHIS documents complement this "Appendix A: Vaccination for Foot-and-Mouth Disease Strategies and Considerations" and provide further details when necessary.

- APHIS Foreign Animal Disease Framework: Response Strategies (FAD PReP Manual 2-0) (April 2014)
- FAD PReP/NAHEMS Guidelines: Vaccination for Contagious Diseases (2014)
- FMD Response: The Red Book Presentation (2014), USDA-APHIS

These documents are available on the Inside APHIS website for APHIS employees, at http://inside.aphis.usda.gov/vs/em/fadprep.shtml, and also at http://www.aphis.usda.gov/fadprep.

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National Animal Health Emergency Management System

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Appendix A: Vaccination for Foot-and-Mouth Disease
Strategies and Considerations

1. PURPOSE

This Appendix is intended to provide relevant information for federal and state officials and other interested parties who will participate in making decisions related to use of vaccine as an aid to control an outbreak of foot and mouth disease (FMD) in the U.S. The following topics are presented and discussed:

- Important characteristics of FMD
- Characteristics of vaccines
- Strategies for vaccine use
- Various factors that must be considered when designing an effective vaccination program

The USDA-APHIS has a separate document, FMD Response Plan: The Red Book, which identifies the capabilities needed to respond to an FMD outbreak in the United States as well as identifying all the critical activities involved in responding with the corresponding time-frames. Please refer to that document for those specific details.

2. BACKGROUND

Recent outbreaks of FMD, particularly the 2001 epizootics in the United Kingdom, the Netherlands, Argentina and Uruguay, have renewed interest in vaccination as a component of control and eradication programs. FMD vaccination is used routinely in endemic areas to protect animals from clinical signs. In a country that is free of this disease, vaccination can be used as an emergency measure to slow virus transmission during an outbreak. It may also decrease the number of animals that must be slaughtered. Foot and mouth disease virus (FMDV) is highly transmissible and can be spread widely by direct contact, as well as in aerosols and on fomites. In some recent outbreaks, the number of animals that had to be destroyed created difficulties with carcass disposal, and raised environmental, ethical and welfare concerns from the public and agricultural communities, as well as causing anxiety and exacerbating other human costs to farming families and others who are dependent on livestock production [1;2]. In particular, the number of apparently healthy animals that were slaughtered in the U.K. and the Netherlands resulted in intense public criticism [1-4]. In 2004, participants in the World Organization for Animal Health (OIE) International Conference on the Control of Infectious Animal Diseases by Vaccination in Buenos Aires, Argentina concluded that mass slaughter is no longer acceptable as the main technique for disease control and eradication, due to ethical, ecological and economic concerns [5]. They recommended that methods for disease prevention, control and eradication be reviewed, and advised an increased emphasis on vaccination.

3. OVERVIEW OF FMD

Summary

The seven serotypes of FMDV (O, A, C, Asia-1, SAT-1, SAT-2 and SAT-3) contain more than 65 strains. Serotype A and the SAT viruses are highly variable, but the Asia-1 viruses have tended to remain relatively stable in their antigenic types (however, new variants have been recognized during recent outbreaks). FMDV strains can vary in their species preferences, clinical presentation, transmission characteristics and possibly their tendency to become established in carriers. It may be difficult to predict the behavior of a field strain of FMDV unless its epidemiology is already known from other epidemics and controlled experiments.

There is no cross-protection between serotypes of FMDV after vaccination with an inactivated vaccine. Within a serotype, protection between strains varies with their antigenic similarity.

FMDV can infect most or all members of the order Artiodactyla (cloven-hooved mammals), as well as a few species in other orders. Cattle are usually the most important maintenance hosts for FMDV; however, African buffalo are important in maintaining SAT type viruses in Africa. It is possible, though unproven, that SAT viruses may not persist long-term outside Africa. Some FMDV isolates may circulate in populations of Asian water buffalo. Certain FMDV strains can primarily be found in pigs, sheep or goats. It is unclear whether small ruminants can maintain FMDV for long periods if other species are absent. Their importance in transmission might vary with the outbreak and region. FMDV does not seem to persist in wildlife hosts (other than African buffalo) for more than a few months, if domesticated livestock are not infected. The potential for feral populations of domesticated animals (e.g., feral swine) or wild relatives of domesticated species to maintain FMDV should be considered in control plans.

The incubation period for FMD can be as short as 18-24 hours, or as long as 14 days in some species. The clinical signs and severity of FMD can vary with the species of animal, and the serotype and strain of the virus. Inapparent or mild infections can occur in sheep, goats and water buffalo, but also in other species under some conditions. High fatality rates have occasionally been reported in some species of wildlife or zoo animals. Among domesticated animals, deaths usually occur mainly in the young.

FMDV can be found in all secretions and excretions from acutely infected animals, and shedding can occur for up to 4 days before the onset of clinical signs. Shedding usually peaks at or near the time when the vesicles rupture and most clinical signs appear.

During an outbreak, vaccination decisions and zones should be based, in part, on the number and species of animals in the outbreak area and surrounding regions. Species vary in the amount of virus shed in various secretions, particularly exhaled air, and in their susceptibility to different routes of infection.

Cattle are especially susceptible to infection by aerosols. Sheep and goats are also susceptible to this route, but their lung volume is smaller and infection during direct contact is thought to be more common. Pigs are relatively resistant to infection via aerosols, compared to ruminants, and there is a possibility that they might not become infected if they are physically separated from infected animals.

Swine herds can produce extensive plumes of aerosolized virus. Sheep produce much less aerosolized virus than pigs, and they are unlikely to transmit FMDV by aerosols farther than 100 meters. A large herd of cattle can produce enough viruses to infect neighboring herds. In one model, the distance FMDV is expected to spread by aerosols varies dramatically depending on the species and number or animals generating the airborne plume, and the species that are exposed downwind.

Airborne transmission is more important for some topotypes and strains of FMDV than others.

Transmission seems to occur less readily between sheep than between cattle or pigs. Even if sheep are not vaccinated, only a proportion of the animals within a herd may become infected.

There is limited information on the survival of FMDV in the environment, but most studies suggest that it remains viable, on average, for three months or less. Virus stability increases at lower temperatures, and in very cold climates, survival up to six months or more may be possible. FMDV can also persist in meat and other animal products, depending on the pH.

FMDV might be carried mechanically in the nares of uninfected humans for short periods. How long the virus can persist is still uncertain, but recent studies suggest that the virus disappears from the nasal passages of most people very soon after exposure.

Effective vaccination can decrease transmission between animals by 1) decreasing the susceptibility of animals to infection, and 2) reducing virus shedding, if a vaccinated animal becomes infected.

3.1 Serotypes and Strains

FMDV is a member of the genus *Aphthovirus* in the family Picornaviridae. As an RNA virus, FMDV has significant genetic variability. There are seven serotypes: O, A, C, Asia-1, SAT-1, SAT-2 and SAT-3. Within these serotypes, more than 65 strains have been recognized [6]. Older strains have names such as O_1 Manisa or A_{24} Cruzeiro, but the names of recently isolated strains are more standardized and include the date and location of isolation (e.g., O/UK/35/2001). Some serotypes have been divided into topotypes, genetically and geographically distinct units that contain closely related strains of the virus [7]. Asia-1 viruses have sometimes been classified into various "groups" or lineages [7;8].

Overall, type O is usually the most prevalent and widely distributed serotype (although serotype A viruses were reported more often in 2013) [8-10]. Serotype O currently contains eight topotypes: Middle East-South Asia (ME-SA), Southeast Asia (SEA), Cathay, Indonesia-1, Indonesia-2, East Africa, West Africa and Europe-South America (Euro-SA) [7]. ME-SA is the dominant topotype, and contains the PanAsia lineage of FMDV. This lineage became prominent in India in the 1990s, spread into most of Asia, and has been responsible for a number of recent outbreaks in FMD-free countries throughout the world [7;11]. In addition to causing the 2001 epizootic in the U.K., the PanAsia lineage affected Taiwan, Japan, South Africa, France, the Netherlands and South Korea in 2000-2002, and caused epizootics in a number of Middle Eastern countries in 2007 [10]. Serotype A is antigenically and genetically diverse, and also contains a number of topotypes [7;9]. Antigenically novel strains of this serotype have emerged and disappeared regularly in Asia and South America [9]. SAT strains are likewise highly variable [9]. Asia-1 viruses have tended to remain relatively stable in their antigenic types, despite the occasional emergence of new strains [9]. However, this serotype has recently caused a number of outbreaks throughout Asia, and appears to have spread rapidly, causing concern [12] New Asia-1 variants, which are poorly matched with the standard vaccine strain (Asia-1 Shamir) have been recognized during these outbreaks [8]. Some FMDV serotypes are rarely seen. SAT 3 is uncommon in domesticated animals (although it can be found in wildlife in Africa), and the last known cases caused by serotype C occurred in Brazil and Kenya in 2004 [8-10;13].

The most common serotypes and strains vary with the geographic region [13]. Globally, FMD viruses have been divided into 7 pools (Eurasia, Eastern Asia, Southern Asia, Eastern Africa, Western Africa, Southern Africa and South America), which seem to maintain distinct groups of viruses [8]. Types O, A, SAT-1, SAT-2 and SAT-3 are the serotypes usually reported from Africa, while serotypes O, A and Asia-1 occur in Asia. FMD viruses frequently enter the Middle East from both Asia and Africa [13]. Serotypes O, Asia-1 and A are common in this region, and SAT-1 and SAT-2 viruses also make periodic incursions from Africa. In the long term, however, the SAT viruses seem able to persist only in Africa [9]. Only serotypes O and A are usually detected in South America [13]. Few outbreaks have been reported from this region in recent years [8]. The predominant FMDV topotypes in a region sometimes remain stable for long periods [9;13]. However, viruses can also spread into new areas, and new strains can develop spontaneously.

Strains of FMDV can vary in their species preferences, clinical presentation, transmission characteristics and possibly their tendency to become established in carriers [11;14-18]. It may be difficult to predict the behavior of a field strain of FMDV unless its epidemiology is already known from other epidemics and controlled experiments [11]. Some high threat lineages, such as Pan-Asia (serotype O), are relatively well

characterized. Viruses of this lineage affected a variety of species including cattle, pigs, sheep and goats in some outbreaks, but they displayed more limited host preferences in others [9].

Animals that have been infected by, or immunized against, one FMDV do not necessarily have immunity to other strains. Conventional inactivated vaccines do not protect animals against other serotypes of FMDV [16;19]. An infection also provides little or no protection against other serotypes, although there are a few reports of apparent cross-protection in cattle, resulting in milder or asymptomatic infections ([20] cited in [19]). Possible explanations for these cases include immune responses to conserved epitopes recognized by CD8+ T cells [21] and/or to conserved nonstructural proteins [19]. Within a serotype, protection between strains varies with their antigenic similarity [16;22].

3.2 Species Affected

FMDV can infect most or all members of the order *Artiodactyla* (cloven-hooved mammals), as well as a few species in other orders. Livestock susceptible to FMD include cattle, pigs, sheep and goats, as well as Asian water buffalo (*Bubalus bubalis*) and reindeer (*Rangifer tarandus)*, which are not farmed extensively in the U.S. Some ranched species including American bison (*Bison bison*) and cervids (e.g., deer and elk [*Cervus elaphus nelsonii*]), are also hosts for the virus. Llamas and alpacas can be infected experimentally, but they do not seem to be very susceptible, and natural infections do not appear to be common [23-26]. While antibodies have been detected at low prevalence in llamas, and infections in alpacas were suspected during one FMD outbreak, there are currently no confirmed cases from the field [23-26]. Recent studies suggest that Bactrian camels (*Camelus bactrianus*) can develop FMD, but dromedary camels (*Camelus dromedarius*) have little or no susceptibility to this virus [25-29]. In 2012, one study reported small numbers of seropositive dromedaries in an endemic region [30]. FMDV is not known to infect horses, mules or donkeys.

At least 70 species of wild or captive wild (e.g., zoo) animals are variably susceptible to FMD [26;31;32]. Most are members of the *Artiodactyla*. Some species reported to be affected include African buffalo (*Syncerus caffer*), American bison , European bison/ wisents (*Bison bonasus*), moose (*Alces alces*), chamois (*Rupicapra rupicapra*), giraffes (*Giraffa camelopardalis*), wildebeest (*Connochaetes gnou*), blackbuck (*Antilopa cervicapra*), waterbuck (*Kobus ellipsiprymnus*), warthogs (*Phacochoerus aethiopicus*), wild boar (*Sus scrofa*), kudu (*Tragelaphus strepsicornis*), impala (*Aepyceros melampus*), tapir (*Tapirus* spp.), gaur (*Bos gaurus*), gayal (*Bos frontalis*), kouprey (*Bos sauveli*), mouflon sheep (*Ovis musinum*), eland (*Taurotragus* spp.), babirusa (*Babyrousa babyrussa*),white-tailed deer (*Odocoileus virginianus*), and several other species of deer, antelopes and gazelles [26;31;32]. Additional species have been infected experimentally or found to have antibodies in nature [26;31;32]. There are no reports of FMD in hippopotamus (*Hippopotamus amphibius*), and serology in South Africa found no evidence of infection in this species [31;32]. Non-cloven-hooved animals reported to be susceptible to natural and/or experimental infection include European and African hedgehogs (*Erinaceus europaeus* and *Atelerix prurei*), armadillos, kangaroos, nutrias (*Myocastor coypus*), chinchillas (*Chinchilla lanigera*), capybaras (*Hydrochaerus hydrochaeris*), mink (*Mustela vison*), European moles (*Talpa europaea*), and voles [26;31-33]. Several cases of FMD have been seen in captive Asian elephants (*Elephas maximus*), but there are few reported infections in African elephants (*Loxodonta africana*), and there has never been any evidence of FMD in this species under natural conditions in Africa [26;31]. Clinical cases have been reported in various species of bears in zoos, including grizzlies (*Ursus arctos horribilis*) and brown bears (*Ursus arctos*) ([34] reviewed in [32]; and [35-37] reviewed in [26]); however, confirmation was lacking until a recent zoo outbreak, when virologically confirmed cases were reported in Tibetan/ Asiatic black bears (*Ursus thibetanus*) [38] There is a report of a fatal FMDV infection in one crested porcupine (*Hystrix cristata*) ([39;40] reviewed in [32]). The diagnosis was made solely by histology, based on myocardial necrosis. Experimental infections in this species were mild ([41] cited in [26]). Laboratory animal models include guinea pigs, rats and mice, but these species are not important in transmitting FMDV in the field [31].

Cattle are usually the most important maintenance hosts for FMDV except in Africa, where African buffalo maintain SAT type viruses [16;42]. There is also evidence that some FMDV isolates might circulate in populations of Asian water buffalo [43;44]. Some viral strains may primarily be found in pigs, sheep or goats [16;45]. The pig-adapted serotype O Cathay strain has not infected large ruminants in outbreaks, and it does not grow in ruminant cells on primary isolation [16]. This strain has been isolated only once from a bovine [46]. When inoculated directly into two cattle, the latter isolate caused only local lesions at the inoculation site in one animal, and there was no evidence of infection in the other animal [46]. A serotype A strain that appeared to be porcinophilic has also been described [45]. Some serotype O strains are well-adapted to sheep and goats, although they can also affect cattle [47]. However, it is uncertain whether small ruminants can maintain FMDV for long periods if cattle are absent [16;47-49]. African buffalo often act as long term reservoir hosts for the SAT serotypes in Africa; there are reports of FMDV maintained in a herd of African buffalo for at least 24 years [14;16]. Some evidence suggests that certain wildlife (or ranched wild animal) hosts might transmit FMDV more readily than others. In one experiment, experimentally infected elk did not seem to spread this virus readily to other elk or to cattle, but transmission did occur between American bison [50]. Experimentally infected white-tailed deer could also infect cattle [51], while giraffe are reportedly unable to transmit FMDV to other giraffe ([52] cited in [26]).

With the exception of African buffalo, there is currently no evidence that wildlife hosts maintain FMDV for more than a few months if domesticated livestock are not infected [16;31;53]. Although early reports suggested that transmission occurred between cattle and European hedgehogs (*Erinaceus europaeus*), there is no evidence that hedgehogs have helped propagate FMDV in recent times [31]. Outbreaks have, however, occurred for short periods among wildlife. In 2011, FMDV was detected in wild boar during outbreaks in Bulgaria [26;54]. Seropositive wild boar and roe deer were later detected, mainly within 15 km around outbreaks in livestock [54]. Although limited virus circulation had probably occurred, there was no virological evidence of infection in any species at this time, suggesting that FMDV had not persisted in wildlife [54]. FMDV has also been isolated from some wild boar sampled in Israel [45]. Some other wildlife affected were impala in South Africa in the late 19th century, and mountain gazelles (*Gazella gazella)* at a nature reserve in Israel in 1985 [31;32;55]. Mortality rates were high in both instances. In 1924-1926, FMDV was speculated to have spread from cattle to wild mule deer (*Odocoileus hemionus*) in California ([56] reviewed in [32]). During this outbreak, lesions consistent with FMD were found in 10% of the deer that were killed. It should be noted that such lesions are also consistent with other cervid diseases, and there is no documentation of any definitive (i.e., laboratory) evidence for FMDV infection in this instance. FMDV did not seem to affect any wild species during some other outbreaks in domesticated animals. There is no evidence that cervids or wild boar were infected or involved in the epidemiology of the 2001 outbreaks in the Netherlands or the U.K. [26]. Water deer (*Hydropotes inermis*), which are among the wildlife most likely to approach farms in South Korea, were seronegative during and after the FMD outbreak in 2010-2011, despite evidence that they had been exposed to some other bovine pathogens [57]. Wild boar and deer were also seronegative after the 2010 outbreak in Japan [58]. Likewise, three serosurveys in deer or European bison (*Bison bonasus*) in Germany and Poland found no evidence of past infection or exposure ([59-61] cited in [26]). Several surveys in South America did not detect any evidence of infection in free-ranging vicuna (*Vicugna vicugna*), guanacos (*Lama guanicoe*), grey brocket deer (*Mazama gouazoubira*), pampas deer (*Ozotoceros bezoarticus celer*) or pudu [26]. Serosurveys in a population of marsh deer (*Blastocerus dichotomus*) in Brazil revealed only low, possibly nonspecific, titers, and PCR tests and virus isolation were negative [62]. The potential for feral populations of domesticated animals (e.g., feral swine) to maintain FMDV should also be considered. Factors that may influence the likelihood of transmission in wildlife include their distribution, social organization, age structure, habitat requirements, home range and any barriers to dispersal [54;63]. Some modeling studies, based on conditions in the U.S. (southern Texas) or Australia, have suggested that outbreaks could occur in wild populations, such as deer or feral swine, and that these animals could theoretically introduce FMDV to domesticated livestock populations

under some circumstances [63-65]. Some assumptions in these models are still untested (e.g., that free range cattle will become infected via fomites or other means if infected wildlife are present in the same area).

3.3 Pathogenesis

FMDV is thought to replicate at a primary site before it disseminates. After infection via aerosols, this location appears to be the nasopharynx in cattle [66] and possibly other ruminants [45]. Replication at the primary site is followed by viremia, usually accompanied by a fever, with dissemination to secondary replication sites [10]. In cattle infected by aerosols, viremia was reported to coincide with the replication of virus in pneumocytes in the lungs and decreased replication in the nasopharyngeal tissues [66]. Viremia lasts 2-3 days, and ends when circulating antibodies appear ([67] cited in [10]). The secondary replication sites for FMDV are primarily stratified, cornified squamous epithelia [14]. Viral replication in skin and mucous membranes at locations such as the mouth, snout, feet and teats causes the formation of vesicles. Although there are some species differences in timing, peak virus production usually occurs around the time the vesicles rupture and most clinical signs appear [14;46;47;68]. In some cases, replication can peak as early as 2-3 days after infection [14]. FMDV is usually eliminated from secondary sites of replication within 10-14 days ([69] cited in [10]). Some ruminants become carriers, defined as the persistence of virus or the viral genome in the pharyngeal region for longer than 28 days.

3.4 Clinical Signs

For official control purposes, the World Organization for Animal Health (OIE) defines the incubation period for FMD as 14 days [70]. In cattle, clinical signs appear in two to 14 days, depending on the dose of the virus and route of infection [71]. In pigs, the incubation period is usually two days or more (with some experiments reporting the appearance of clinical signs as early as 18-24 hours), and may be as long as 9 days [46]. Clinical signs usually develop in 3-8 days in sheep, although they can appear as quickly as 24 hours or as long as 12 days after experimental infection [14;47;72]. Other reported incubation periods are 4 days in wild boar, 2 days in feral pigs, 2-3 days in elk, 2-14 days in Bactrian camels, and possibly up to 21 days in water buffalo infected by direct contact [25;29;50;73-75].

While there is some variability in the clinical signs between species, FMD is typically an acute febrile illness with vesicles developing in limited locations, usually on the feet, in and around the mouth, and on the udder [23;31;46;47;71;76]. Occasionally, they may be found at other sites including the vulva, prepuce or pressure points on the legs. The vesicles often rupture rapidly, becoming erosions. Pain and discomfort from these lesions leads to a variety of clinical signs such as depression, anorexia, excessive salivation, lameness and reluctance to move or rise. Lesions on the coronary band can cause growth arrest lines on the hoof. In severe cases, the hooves or footpads may be sloughed. Reproductive losses are possible, and have been reported mainly in small ruminants [45;77]. Most adult animals recover within two to three weeks, although secondary bacterial infections may lead to a longer recovery time [23;76]. Among domesticated animals, deaths usually occur mainly in the young, as the result of multifocal myocarditis (vesicles are not always found in these cases) or starvation [16;23]. In some outbreaks, the mortality in young animals can be very high [22;23;46;47;71]. Although severe FMD may also cause deaths among older animals, the mortality rate is usually 1-5% among adult livestock after natural infections with most strains [76]. High fatality rates have occasionally been reported in some species of wildlife or zoo animals [31;32].

3.4.1 Species Differences in Clinical Signs

The clinical signs and severity of FMD can vary with the species of animal, and the serotype and strain of the virus. This may affect how readily the illness is recognized. In highly productive beef and dairy breeds, such as those found in North America, clinical signs are usually apparent [71]. Although the first cases in a herd may be mild or even subclinical if the exposure is low, cattle infected after the virus has been circulating in the herd tend to be severely affected [71]. Cattle typically become febrile and develop lesions on the tongue, dental pad, gums, soft palate, nostrils or muzzle, and sometimes the teat [23;71;76].

Hoof lesions occur in the area of the coronary band and interdigital space, and can cause lameness, reluctance to rise, or stamping or shaking of the feet. The loss of condition can cause a drop in milk production, which does not usually recover during that lactation. Secondary mastitis may also be seen. Indigenous cattle breeds in Asia and Africa where FMD is endemic tend to have much less severe clinical signs [71]. In addition to other complications such as mastitis or hoof malformations, some cattle that recover from FMD are reported to develop heat-intolerance syndrome (HIS; also called 'hairy panters') [45]. This poorly understood syndrome is characterized by abnormal hair growth (with failure of normal seasonal shedding), pronounced panting with elevated body temperature and pulse rate during hot weather, and failure to thrive. Some affected animals are reported to have low body weight, severely reduced milk production and reproductive disturbances. Animals with HIS do not seem to recover. The pathogenesis of this syndrome is not known, and a definitive link with FMD has not been established, but infection-associated endocrine disturbances were suspected by some early investigators.

In pigs, the most severe lesions usually occur on the feet [23;46;76;78]. The snout and udder may also be affected, and lesions may be seen on the hock and elbow if the animals are on rough concrete. Mouth lesions are typically small and less apparent than in cattle, and drooling is rare. Fever may be seen in pigs, but the temperature elevation can be short or inconsistent [46]. Lesions may be less apparent in feral pigs than domesticated pigs, in part due to their thicker skin and long, coarse hair, although the severity of clinical signs seems to be similar [73]. In one experiment, clinical signs were milder in wild boar [74].

In contrast to pigs and cattle, the clinical signs in sheep and goats tend to be mild [23;47;76;78;79]. The signs can vary with the virus. The most common clinical signs are fever and mild to severe lameness of one or more legs. Vesicles can develop in the interdigital cleft and on the heel bulbs and coronary band, but they may rupture and be hidden by foot lesions from other causes. Mouth lesions are often not noticeable or severe in sheep, and generally appear as shallow erosions. Nevertheless, there are reports of experimental infections where foot lesions were less prominent than oral lesions [45]. Approximately 25% of infected sheep remain asymptomatic, and 20% have a single lesion [47]. In different experimental or field reports, clinical signs in goats were reported to be either more or less apparent than in sheep [45]. Reproductive losses have been reported more frequently in small ruminants than in other species [45;77].

Some studies have reported that the clinical signs tend to be milder in water buffalo than in cattle, and lesions may heal more rapidly [75;80;81]. Although both mouth and foot lesions can occur in this species, some studies reported that mouth lesions were smaller than in cattle, with scant fluid [81;82]. Nasal discharge may be copious. One group reported that foot lesions were more likely to occur on the bulb of the heel than in the interdigital space of experimentally infected animals [81].

Experimentally infected llamas and alpacas are generally reported to have only mild clinical signs, or to remain asymptomatic, although some reviews indicate that severe infections can also occur [23;25]. Mild signs were reported in alpacas during one FMD outbreak in Peru, but the virus could not be isolated and these cases are unconfirmed [25]. There are no reports of natural infections in llamas [25]. Two experimentally infected Bactrian camels developed moderate to severe clinical signs, with hindleg lesions including swelling and exudation of the footpad, but no oral lesions [29]. Mouth lesions and salivation, as well as severe footpad lesions and skin sloughing at the carpal and tarsal joints, the chest and knee pads were reported from Bactrian camels during outbreaks in the former Soviet Union [25]. Detachment of the soles of the feet has been noted in several reports [25].

The clinical signs in wildlife resemble those seen in domesticated livestock [31]. Vesicles and erosions may be found at various sites, particularly on the feet, and in and around the mouth. More severe lesions occur where there is frequent mechanical trauma, e.g. on the feet and snout of suids or the carpal joints of warthogs. Loss of horns has also been seen. Some wildlife species typically experience subclinical infections or mild disease, while others develop severe, acute illness. Infections with SAT-type viruses in

African buffalo are often subclinical, although small mouth and/or foot lesions have been reported. South American pudu (*Pudu pudu*) seem to be highly susceptible; during an outbreak at the Cologne Zoo in Germany, no other deer were affected but 5 of 8 pudu died ([40] reviewed in [32]). Severe illness has also been documented in a population of mountain gazelles, as well as in impala, blackbuck, some white tailed-deer, saiga antelope (*Saiga tatarica*), warthogs, a kangaroo and some other species [26;31;32;45] Young animals of any species can die suddenly of myocarditis [31].

3.5 Transmission

FMDV can be found in all secretions and excretions from acutely infected animals, including expired air, saliva, nasal secretions, lachrymal fluid, milk, urine, feces and semen [14;16;76]. In sheep, it has also been demonstrated in amniotic fluid and aborted fetuses [77]. Animals can shed the virus for up to four days before the onset of clinical signs [71]. FMDV also occurs in vesicle fluid, and large quantities of virus may be shed when the vesicles rupture [14;46;47;68]. There are some species differences in the timing of virus shedding: in sheep, maximal virus excretion may occur 1-2 days before the animals develop clinical signs, while in cattle and pigs, maximal shedding is around the time of vesicle formation [9;15]. One study reported that the estimated transmission rates from subclinically infected animals, including animals incubating the disease, were low or relatively low in infected (nonvaccinated) lambs and calves, but much higher in piglets and dairy cattle [83]. FMDV can be transmitted to other animals by direct contact, or by indirect contact via aerosols or contaminated fomites and environments [16;23]. Possible routes of entry into the body include inhalation of aerosolized virus, ingestion of contaminated feed, and entry of the virus through skin abrasions or mucous membranes [46;47;71]. The importance of each of these routes varies with the species (see below). Sexual transmission can occur, and could be a significant route of spread for viruses of the SAT serotype [14;23;31;84]. In sheep, FMDV has been shown to cross the placenta and infect the fetus [77].

The amount of aerosolized virus produced varies with the strain of FMDV, and airborne transmission is thought to be more important for some topotypes and strains than others [11;15]. For example, the C Noville strain is infectious over distances that may be up to 50 times greater than for a strain of the Pan-Asia lineage of serotype O [11]. In some locations, there seems to be little or no aerosol transmission of pig-adapted O Cathay viruses between herds [9]. Airborne transmission is influenced by climatic conditions, and FMDV also spreads much farther over water than land [85]. One viral strain is thought to have been transmitted via aerosols from Brittany, France to the Isle of Wight, U.K. in 1981, a distance of more than 250 km [11]. Aerosol transmission over land alone is said to be rarely greater than 10 km [11]; however, greater distances are sometimes reported under favorable conditions. In the 2001 epizootic in the U.K., airborne transmission of 16 km was reported from one farm when atmospheric conditions were very stable, up to 300 infected cattle were producing FMDV, and the virus traveled over a smooth river estuary [86]. An airborne plume was reported to spread the virus 60 km during the 1967-68 outbreak in the U.K [15].

There is limited information on the survival of FMDV in the environment, but most studies suggest that it remains viable, on average, for three months or less [87]. Virus stability increases at lower temperatures, and in very cold climates, survival up to six months or more may be possible. Like other viruses, survival is enhanced by the presence of organic material and protection from sunlight. FMDV was reported to survive on bran and hay for more than three months in a laboratory, on wool at 4°C for approximately two months (with significantly decreased survival at a temperature of 18°C/ 64°F), and in bovine feces for 2-3 months [87]. In a recent study, complete loss of viability under anaerobic conditions at 20°C required 14 days in pig slurry or 21 days in bovine slurry, while viruses in either pig or cattle slurry were inactivated in 48 hours or less at temperatures of 35°C or higher [88]. Inactivation was still incomplete in pig slurry after 14 weeks at 5°C [88]. FMDV is also inactivated at pH below 6.0 or above 9.0 [76]. It can persist in meat and other animal products when the pH remains above 6.0, but it is inactivated by the acidification of muscles during rigor mortis [9;76;89]. However, acidification does not occur to this extent

in the bones and glands, and FMDV may persist in these tissues [9]. In addition, the acidity in meat from some animals (e.g., stressed cattle, febrile FMDV-infected sheep) may not reach levels necessary for destroy the virus [90]. FMDV may also persist in meat that is frozen soon after slaughter, although the pH drop that occurs after thawing can later inactivate it [90].

Seasonal changes in animal movements and trading patterns can lead to seasonality in FMD outbreaks [91], or to an increased risk of epizootics from an introduced virus at certain times of the year [92].

People can act as mechanical vectors for FMDV, by carrying the virus on clothing or skin. The virus might also be carried for a brief period in the nasal passages, although several studies suggest prolonged carriage is unlikely. In one study, this virus was detected in the nasal passages of one of eight people 28 hours after exposure to infected animals, and from none of the eight at 48 hours [93]. "No contact" periods for responders in FMD outbreaks have been based on this study. More recent research found that people did not transmit serotype O FMD viruses (O/UK/35/2001 and O/TAW/97) to pigs or sheep when personal hygiene and biosecurity protocols were followed, and suggested that nasal carriage of the virus might be unimportant in transmission [94;95]. In one of the latter studies, virus was detected in the nasal secretions of one of four people immediately after contact with infected animals, but it was not found in samples taken between 12 and 84 hours [94]. In the second study, FMDV was not isolated from the nares [95]. Another study, which used PCR to detect FMDV nucleic acids, also suggests that persistent nasal carriage is uncommon. In this study, viral nucleic acids could be detected in nasal samples from only one of 68 people, 16-22 hours after close contact with infected research animals (sheep, cattle and pigs infected with the viruses Asia-1 HKN 5/05, O UKG 34/ 2001 and O BFS 1860/67) in a closed environment, although a number of nasal samples tested positive by PCR immediately after exposure [96]. Virus could not be isolated from the single PCR-positive sample. No nasal samples contained FMDV nucleic acids in three other experiments, when people were tested the day after exposure [96]. Eight people were tested 2-3 days after exposure, and no PCR-positive nasal samples were found [96]. However, it is possible that results might be different with other strains or serotypes of the virus. Factors such as intensive contact between people and animals, high pathogen loads, highly susceptible animals, sub-optimal facility sanitation or poor compliance with personal hygiene and biosecurity protocols could influence transmission in the field.

3.5.1 Vaccination and Virus Transmission

Effective vaccination can decrease transmission between animals by 1) decreasing the susceptibility of animals to infection, and 2) reducing virus shedding, if a vaccinated animal becomes infected. Details are available in section 10.

3.6 Species Differences in Transmission That May Affect Vaccination Decisions

FMDV can be transmitted by multiple routes, and species vary in the amount of virus shed in various secretions, particularly exhaled air. They also vary in their susceptibility to different routes of infection. During an outbreak, vaccination decisions and zones may need to be based, in part, on the number and species of animals in the outbreak area and surrounding regions.

3.6.1 Cattle

Cattle can become infected through breaks in the skin or mucous membranes, or via aerosols [71]. Because cattle have a large respiratory volume, and the infectious dose by inhalation may be as low as 20 $TCID_{50}$, they are particularly susceptible to infection by aerosols [71]. Airborne FMDV may infect this species either from nearby animals or over longer distances. Calves could also acquire the virus by insufflation of milk. Cattle can be infected by ingestion; however, the infectious dose may be as much as 10,000 times greater than by inhalation [71]. Cattle generate up to $\log_{10}$ 5.1 $TCID_{50}$ of aerosolized virus per day, and a large herd can produce enough viruses to infect neighboring herds [71]. Peak shedding of up to $\log_{10}$ 6.7 $TCID_{50}$ per ml occurs in milk, with as much as $\log_{10}$ 6.2 $TCID_{50}$ per ml in semen, $\log_{10}$ 4.9

$TCID_{50}$ per ml in urine and $\log_{10}$ 5.0 $TCID_{50}$ per ml in feces [71]. Milk and semen can contain virus up to 4 days before the clinical signs appear. In a nonvaccinated cattle herd, transmission usually occurs quickly; most animals become infected, and most may have developed clinical signs by the time the herd is diagnosed [71]. Often, 90% of the herd may eventually be affected [9]. For this reason, infected herds tend to be detected by clinical signs if they are fully susceptible.

3.6.2 Sheep and Goats

Similarly to cattle, the infectious dose in sheep and goats can be as little as 20 $TCID_{50}$; however, their lung volume is smaller, and they may be less susceptible to infection by aerosols [47]. Direct contact is probably a more common route of infection in small ruminants. They may acquire the virus through abrasions in the skin and mucous membranes, by ingestion, or via inhalation from nearby animals [47]. Transmission between nonvaccinated sheep seems to occur less readily than between cattle or pigs [47;49]. In some cases, only a small percentage of the flock becomes seropositive or sheds virus [47]. There are cases where only 25% of the animals were infected before the virus disappeared from the flock [9]. Sheep produce much less aerosolized virus than pigs, and they are unlikely to transmit FMDV by aerosols farther than 100 meters [47]. Transmission can occur subclinically in sheep flocks, or with limited lesions, and there is a significant danger that infected flocks might not be detected [47].

It is still uncertain whether small ruminants can maintain FMDV in the absence of other infected species [16;47;49]. There is limited field and experimental evidence that some, and possibly most, strains might die out during serial passage in these animals. However, definitive evidence is lacking, and one recent study found a reproduction ratio of 1.14 among nonvaccinated sheep [49]. Two studies reported prolonged shedding in lambs, compared to calves, using an Asia-1 strain (TUR/11/2000) [97;98] Despite this, virus transmission from lambs to calves occurred at a relatively low rate [97], and transmission to contact lambs only occurred during the first week after inoculation, when virus titers were highest [98]. The importance of small ruminants in transmission might vary with the outbreak and region. In some endemic areas, only minor outbreaks occur in these animals; in other regions, the seroprevalence is high in sheep and goats, but outbreaks are not seen in other species ([48;99] cited in [49]). Sheep are thought to have been important in spreading FMDV inapparently during the early stages of the 2001 FMD epizootic in the UK, and may also have been important in other outbreaks, including the 1999 epizootic in Morocco ([100;101] cited in [49]). In contrast, the epidemiology of FMD in endemic areas of Kenya, as well as outbreaks in Uruguay, Greece and North Africa suggests a minor role for this species ([48] cited in [49]). In 2007, serological and epidemiological evidence suggested that sheep and goats in Cyprus had been infected with FMDV three years earlier, but the virus had died out without causing clinical signs or affecting cattle or pigs [102]. It is possible that the behavior of the virus in small ruminants varies with the species adaptation of the strain and/or epidemiological factors. Infected cattle or pigs can raise the amount of FMDV in the environment and increase the prevalence in nearby sheep herds [47].

3.6.3 Pigs

Pigs are usually infected by direct contact with infected animals or heavily contaminated environments, or by ingestion of the virus [46]. The infectious dose might vary with the individual pig, and possibly the strain of FMDV. The oral infectious dose has been estimated to be approximately 10^5 $TCID_{50}$, and possibly lower if the animal has mouth lesions. Recently, 10^3 $TCID_{50}$ was reported to be sufficient for oral infection with a Japanese serotype O virus [103]. Pigs are generally reported to be relatively resistant to infection via aerosols, compared to ruminants [15;17;46;103]. In experiments, this species requires up to 6,000 $TCID_{50}$ (as much as 600 times the aerosol dose for cattle or sheep) to become infected by this route. Some field and experimental studies suggest that pigs might not become infected if they are physically separated from infected animals [46;104;105]. Once the virus enters the herd, however, it may spread rapidly, and transmission can occur by inhalation as well as other routes. Often, 90% of the herd is eventually affected [9]. Swine herds can produce extensive plumes of aerosolized virus [15;85]. This species sheds large amounts of FMDV in respiratory secretions, and can produce as much as $\log_{10}$ 5.8 $TCID_{50}$ to $\log_{10}$ 7.6 $TCID_{50}$ per pig in 24 hours [46]. The amount of aerosolized virus varies with the

strain. In a worst case scenario, one model predicts that 1,000 infected pigs could produce an airborne plume of virus that could infect cattle up to a distance of 20-300 km (with the distance depending on the viral strain), sheep up to 10-100 km, and pigs for less than 1 km [15]. If only 100 pigs are infected, cattle are predicted to be susceptible up to 6-90 km away. In contrast, the plume generated by 100 infected cattle or sheep is expected to infect cattle at a distance of less than 1 km.

At low doses of virus, pigs can be subclinically infected, with no clinical signs, viremia that is undetectable or transient and very low, and a brief and low titered immune response [46]. These animals might transmit FMDV inefficiently or not at all. Mild disease is also possible but rare.

4. CARRIERS

Summary

FMDV carriers have been defined as those animals in which virus or viral RNA can be detected for more than 28 days after infection. For FMD, the definition of "carrier" includes animals that may or may not be able to transmit the infection. FMDV persists mainly in the pharyngeal region, and is detected by testing esophageal-pharyngeal fluid. Detection may be intermittent, and the quantity of virus is usually low and declines with time.

Domesticated ruminants known to become carriers include cattle, sheep, goats and water buffalo, but not pigs. Persistent infections do not seem to occur in camelids. Among wildlife, only African buffalo seem to be important as carriers, although FMDV can be recovered for a limited period in some experimentally infected wildlife including some species of deer.

How long an animal remains a carrier varies with the individual animal and the species. African buffalo can carry the virus up to five years. Most cattle carry FMDV for six months or less, but there are reports of persistent infections in this species for up to 3.5 years. Persistent infections have been reported in some water buffalo for up to a year. Most carrier sheep appear to carry FMDV for only 1 to 5 months, although the virus may persist in some individuals for up to 12 months. The longest reported carriage in goats is four months. Whether the length of the carrier state varies with the FMDV strain is poorly understood.

The epidemiological significance of carriers among domesticated livestock is controversial. Unequivocal evidence for transmission from carriers has been reported only for the SAT viruses in African buffalo. Transmission from carrier African buffalo to cattle seems to be inconsistent and sporadic. It is possible that sexual transmission is involved. Carrier cattle may also be able to transmit SAT viruses. One report of live virus in the nasal secretions of water buffalo, 70 days after inoculation, raised the possibility that carriers might be epidemiologically important in this species. In contrast, there is no definitive evidence for the transmission of viruses of serotypes A, O, Asia-1 or C from carrier cattle, sheep or goats, although anecdotal reports suggest that carriers might have been involved in some historical recrudescent outbreaks. Controlled experiments have been uniformly unsuccessful in attempting to demonstrate transmission from domesticated animal carriers by direct contact. The occurrence of carriers does not seem to have interfered with eradication efforts that used vaccination, such as the vaccination campaigns in South America.

The risk that carriers will transmit FMDV is likely to be influenced by the prevalence of carriers in the population. The percentage of animals that become carriers, with or without vaccination, is still uncertain and estimates vary widely. In general, it appears that animals exposed to greater quantities of virus are more likely to become carriers. Some experimental studies also suggest that vaccination may decrease the percentage of carriers by reducing exposure to the virus. Carriers seem to be more common when animals are exposed very soon after they receive the vaccine; however, some experiments suggest

that highly potent vaccines might reduce or prevent carriage in sheep even when homologous or heterologous challenge occurs as soon as 4 days.

FMDV carriers have been defined as those animals in which virus or viral RNA can be detected for more than 28 days (4 weeks) after infection [106]. For FMD, the definition of "carrier" includes animals that may or may not be able to transmit the infection (see below). Animals can become carriers whether or not they develop clinical signs [14]. FMDV persists mainly in the pharyngeal region (although the exact location where it persists and replicates is still unclear [107]) and possibly at other sites [14;42;108]. It is not certain whether this virus is cell-free, possibly in immune complexes, or if it is cell-associated [14]. Carriers can be identified by detecting FMDV in oropharyngeal (probang) samples of esophageal-pharyngeal fluid. Detection may be intermittent, and the quantity of virus is usually low and declines with time [1;14].

How long an animal remains a carrier varies with the individual animal and the species. Most cattle carry FMDV for six months or less, but there are reports of persistent infections in this species for up to 3.5 years [1;14;16;84;109]. Persistent infections have also been reported for up to a year in some water buffalo [6], and for as long as 8 months in yaks (*Bos grunniens*) [110]. Some studies suggest that carriage might be more common in water buffalo than cattle [80]. Sheep and goats seem to become carriers less often, and for a shorter time [84]. Most sheep appear to carry FMDV for only 1 to 5 months, although the virus may persist in some individuals for up to 12 months [84;111]. The longest reported carriage in goats is four months [1;14;111]. Llamas and Bactrian camels do not seem to become carriers [25;42;84]. Limited studies suggest that the establishment of carriers might vary with the strain and serotype of the virus, and possibly the breed of the animal; however, this question is still open, and the length of the carrier state for various FMDV strains is poorly understood [1;14;42;84].

The current consensus is that pigs do not become carriers [14;42;84]. Nevertheless, a few reports have demonstrated prolonged persistence of viral RNA in this species, especially in lymphoid and pharyngeal tissues ([112] cited in [113]; and [45;73;114;115]). Mezencio et al. reported FMDV RNA in the blood of recovered pigs, and fluctuating virus neutralization activity associated with these episodes [114]. However, a recent study could not detect viral RNA in the serum more than 14 days after inoculation, or infectious virus after 10 days [116]. One group reported viral RNA in the lymph nodes and tonsil, but not other tissues in the pharyngeal region, on day 28 [115], while another group found viral RNA, without evidence for live virus, in the tonsils at least until day 33-36 [73]. In wild boar, viral RNA was detected in lymphoid tissues, oropharyngeal fluid and affected areas of the skin on day 28 [74]. One study found that a portion of the viral genome could be amplified from the tissues of the pharynx and dorsal and ventral soft palate of 4 infected pigs after 28 days, but live virus could not be recovered from any tissues, and a probe for the 3D region of FMDV did not detect viral RNA [113]. The authors suggest that a residual portion of the FMDV genome, which degrades slowly, may account for the reports of "carriers" among pigs when tested by PCR. Other authors have suggested alternative explanations, such as the interference of neutralizing antibodies with virus isolation [74]. To date, there have been no reports of virus isolation from pigs or wild boar after 28 days [45;73], although infectious virus has been detected in the oral fluids of some feral swine up to 14 days after inoculation [73], and in lymphoid tissues, including the tonsil, of domesticated pigs as long as 17 days [116].

Among wildlife, virus persistence is reported to be common only in African buffalo. Individual African buffalo have been shown to become carriers for up to five years, with a peak prevalence in 1-3 year old animals [84]. Most young buffalo seem to become infected when they are 2-6 months old, when maternal antibodies have decreased. Persistent infections have been reported for a limited period in some experimentally infected wildlife including fallow deer (*Dama dama*), sika deer and kudu, and occasionally in red deer (*Cervus elaphus*) [31;84]. One study reported that FMDV could rarely be found in red deer or roe deer (*Capreolus capreolus*) after 14 days, but the virus persisted in fallow deer for at

least 5 weeks after infection [84]. It could be found for up to 57 days in sable antelope (*Hippotragus niger*) and for nearly 5 months in kudu [84]. One older study reported finding FMDV for up to 5 weeks in several white-tailed deer, with one deer carrying the virus for 11 weeks [51]. In contrast, a recent study found no evidence for persistent infections in this species: viral RNA was last detected in probang samples 21 days after inoculation, and no virus was isolated at this time [117]. One report suggested that a SAT-1 virus persisted in two wildebeest for 45 days after infection, but this was not confirmed in a later study [84]. There is no evidence for carriers among impala, which are commonly affected by outbreaks in southern Africa [84]. In one early study, experimentally infected brown rats (*Rattus norvegicus*) were carriers for 4 months ([118] cited in [26]).

4.1 Can Carriers Transmit the Virus to Other Animals?

The epidemiological significance of carriers among domesticated livestock is controversial [14;84;119]. Unequivocal evidence for transmission from carriers has been reported only from southern Africa, where African buffalo can spread SAT viruses to other buffalo and have occasionally infected cattle [14;31;84]. Transmission from buffalo to cattle seems to be inconsistent and sporadic. In one study, cattle maintained for 2.5 years with buffalo did not become infected, although the virus was transmitted within the buffalo population ([120] reviewed in [84]). Likewise, 16 experimentally infected African buffalo had not infected 4 cattle in close contact, in an ongoing study as of 2012 (unpublished results by Charleston, 2012 cited in [121]. In another study, SAT-2 virus was sometimes transmitted from African buffalo to both buffalo and cattle in the same enclosure, but this took months in some cases, and the trigger for transmission was unknown ([122] reviewed in [84]). In both this report and an earlier one ([123] reviewed in [84]), male buffalo were present and the cattle were cows, and in the unsuccessful experiments in both cattle and buffalo, there were no bulls. SAT viruses have been detected in semen and sheath washes from persistently infected African buffalo [124]. For these reasons, some authors speculate that sexual transmission might be involved. There are also reliable reports of transmission from buffalo carriers to cattle in the field in Africa [14;31;125]. However, the incidence seems to be low, unless the animals are in close contact. For example, there no evidence that FMD occurred in domesticated animals in Botswana for 8 years, although the virus has been found in 50-70% of wild African buffalo in that country [14;84]. Carrier cattle may also be able to transmit SAT viruses [14;42]. Transmission was reported between cattle carrying SAT-2 viruses after outbreaks in Zimbabwe in the 1980s [42]. In one case, there was no evidence of transmission from cattle to young animals on the farm where the carrier cattle resided, but transmission occurred after the carriers were moved and mixed with other cattle. It is possible that the stress of the movement might have reactivated the virus. Overall, there appears to be a significant risk of transmission from carrier African buffalo, and possibly from cattle to cattle, of SAT viruses [14;42;84]. One group reported the isolation of a serotype O FMDV from the nasal fluid of some experimentally infected water buffalo at 70 days, suggesting that virus carriage might be epidemiologically important in this species [75].

In contrast, there is no definitive evidence for the transmission of serotype A, O, Asia-1 or C viruses from carrier cattle, sheep or goats, although anecdotal reports suggest that carriers might have been involved in some historical recrudescent outbreaks [14;42;84;126]. These incidents include outbreaks in Denmark in 1883-1894 and the UK from 1922 to 1924, as well as an unpublished report of recrudescence in Denmark in 1982-1983 [14]. Most reports of potential transmission from carriers occurred before vaccination was introduced in the 1960s and at a time when a high proportion of animals became persistently infected [84]. Controlled experiments have been uniformly unsuccessful in attempting to demonstrate transmission from carriers by direct contact, although oropharyngeal fluid from carriers can transmit FMDV if it is injected directly into cattle or pigs [14;42;84;127;128]. Some published experiments treated animals with dexamethasone, stressed them, or co-infected them with bovine herpesvirus-1 (infectious bovine rhinotracheitis virus) or rinderpest [14;42;84]. Corticosteroid treatment appears to actually decrease the amount of virus in probang samples from carriers [42;84]. Similarly, trauma to the feet or infection with *Ascaris* spp. was unable to increase the susceptibility of pigs to infection from carriers [84]. Nevertheless,

the possibility of transmission from carrier cattle or small ruminants cannot be definitively excluded [14]. It is possible that the lack of transmission in experimental studies is caused by other factors, such as the good condition of the animals or the absence of sexual activity [127]. The limited number of animals and viral strains that have been used in these experiments might also be a factor [126].

If transmission occurs from carrier cattle, sheep or goats, it has apparently not interfered with eradication efforts using vaccines. Vaccination programs in South America were able to eradicate FMD when good quality vaccines were used in cattle, even when susceptible calves and nonvaccinated sheep and pigs were exposed [129;130]. A small number of carrier animals probably did exist, but virus transmission did not seem to occur, as determined by serological assays, the use of sentinel nonvaccinated animals, and the absence of infections in calves and other susceptible species [129;130]. It should be noted that, in South America, continued use of prophylactic vaccination might have mitigated the risks (if any) from carriers [131]. Vaccination was also a component of FMD eradication in the past in Europe and Mexico, and it has recently been a part of successful eradication programs during outbreaks in Albania, Macedonia and other countries [132;133]. In 2002, a report from the Royal Society, London concluded that the scientific evidence for FMDV transmission from domesticated animal carriers is weak, and that if it occurs, it is very infrequent and happens under a particular (yet unknown) set of circumstances ([134] cited in [3]). According to the Royal Society, the risk of carriers should not preclude the use of emergency vaccination; however, there should be protocols for monitoring vaccinated animals after the epidemic has ended.

4.2 The Effect of Vaccination on the Prevalence of Carriers

The risk that carriers will transmit FMDV is likely to be influenced by the prevalence of carriers in the population. The percentage of animals that become carriers, with or without vaccination, is still uncertain and estimates vary widely. One complication is that experimental studies use a variety of strains, varying routes of inoculation and severity of challenge, and different vaccination protocols. Carriers can be difficult to detect, and some studies may assay for virus carriage at only a few time points (sometimes only one or two). It is also difficult to extrapolate laboratory studies to the field situation where there are larger numbers of animals, and the environment and other conditions are uncontrolled. However, it appears that animals exposed to greater quantities of virus are more likely to become carriers. Studies from the late 1950s and early 1960s, when vaccination campaigns had not yet reduced the incidence of the disease, found that up to half of the recovered cattle in endemic counties were carriers [84]. This was generally the case for all seven FMD serotypes. Early studies also suggested that 50% of sheep became carriers, whether or not they were vaccinated ([135] cited in [49]). In contrast, a survey in Asiatic Turkey in the early 1990s reported that the prevalence of carriers among cattle and sheep was 15–20% ([136] cited in [14]). In Brazil, more than 50% of cattle were carriers in the early 1960s before vaccination was common [84]. This number was greatly reduced by intensified vaccination campaigns, and very few carriers were found in endemic areas by the mid-1980s [84]. In Kenya, the prevalence of carriers in the 1970s was 0.5% in an area where vaccination was practiced, and 3.3% in a region where it was not ([99] cited in [84]).

Some experimental studies also suggest that immunization with an effective vaccine may decrease the percentage of carriers, by reducing exposure to the virus [84;137-146]. Most studies have been conducted in sheep. Barnett et al. reported that increased vaccine potency was correlated with decreased virus replication in the oropharynx and a lower rate of virus carriage among sheep [138]. In this experiment, the two highest doses of a potent vaccine completely prevented the animals from becoming carriers. Madhanmohan et al. likewise reported a dose-dependent decrease in the number of sheep and goat carriers, with no carriers occurring at the highest antigen doses [146]. In an earlier study by this group, 26% of 23 vaccinated sheep and all 6 nonvaccinated sheep became carriers when challenged after 3 weeks [142]. Another laboratory reported virus carriage in nine of 12 nonvaccinated lambs inoculated directly with FMDV and three of 12 nonvaccinated lambs exposed by contact with infected animals, but only one of 24 vaccinated lambs exposed by contact or inoculation [49]. A heterologous challenge

experiment demonstrated that a highly potent vaccine could also reduce the prevalence of carriers (from 53% to 0%) when sheep were challenged with a different virus, 4 days later [144]. However, there was no reduction in the number of sheep carriers detected by PCR (approximately 60%) in a challenge experiment using a poorly matched ($r_1 < 0.3$) heterologous Asia-1 vaccine [98]. In lactating dairy cows, persistent infections were reported in 3 of 10 vaccinated cattle, and 6 of 8 surviving nonvaccinated animals, after direct inoculation with FMDV [140]. Among calves, virus carriage occurred in 3 of 12 virus-inoculated, vaccinated animals, no contact-exposed, vaccinated animals, 5 of 12 virus-inoculated, nonvaccinated calves and 3 of 12 contact-exposed, nonvaccinated calves [139]. Another study in cattle found that immunization with a highly potent vaccine did not reduce the number of carriers after a severe direct contact challenge [147].

In general, carriers seem to be more common when animals are exposed very soon after vaccination. Parida et al. found that 10% of sheep challenged 10 days after vaccination with a highly potent FMD vaccine, and 20% of the animals challenged 4 days after vaccination became carriers, while 37.5% of nonvaccinated sheep were persistently infected [143]. Other authors have also reported that more cattle became carriers if challenged soon after vaccination (e.g., 4 or 6 days), compared to animals challenged at later time points [121;137]. Nevertheless, some highly potent vaccines appear to be able to eliminate carriage among sheep, at least under experimental conditions, as early as 4 days after immunization [144].

5. DETECTION OF INFECTED ANIMALS

Summary

Virological tests used to detect acutely infected animals include ELISAs or lateral flow devices to detect viral antigens, virus isolation and RT-PCR.

Serological tests for FMD can be used to confirm suspected cases, monitor the efficacy of vaccination, and provide evidence for the absence of infection. Serological assays can detect antibodies to either structural (capsid) proteins (SPs) or non-structural proteins (NSPs). Infected animals develop antibodies to both SPs and NSPs, but seroconversion to SPs occurs earlier, and the titers are higher. Antibodies to SPs may also persist longer.

Serological tests based on SPs are serotype-specific. They are highly sensitive if closely matched to the field virus. Their disadvantage is that a single assay cannot be used to detect antibodies to field viruses of different serotypes, or to screen for infections with viruses of unknown serotype. Tests that detect antibodies to SPs cannot determine whether these antibodies are the result of infection or vaccination. OIE-recommended serological SP assays include the virus neutralization test, the solid-phase competition ELISA and the liquid phase blocking ELISA.

Because vaccination primarily induces antibodies to SPs, tests that detect titers to NSPs can recognize infections with field viruses in vaccinated animals. Insufficiently purified vaccines can contain low levels of NSPs, and may induce low titers to these proteins. Vaccine purity is especially important when animals must be vaccinated multiple times.

Serological tests based on NSPs can identify infections in either vaccinated or nonvaccinated animals. Because NSPs are conserved across serotypes and strains, a single assay can recognize infections with all FMD viruses. These tests might not detect animals with limited virus replication, including some vaccinated animals or nonvaccinated, subclinically infected animals. Although these tests have limitations in identifying individual animals, they are valuable as herd tests, and can be used as part of the procedure to regain FMD free status.

In the OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals, the recommended NSP assays are ELISAs and immunoblot assays such as the enzyme-linked immuno-electrotransfer blot

(EITB). In cattle, the NSP proteins 3ABC and 3AB seem to be the most reliable markers to distinguish vaccinated animals from those infected by field strains. Most NSP ELISAs are based on 3ABC. A number of commercial 3ABC ELISA kits, as well as some "in house" tests, are available. Other NSP assays have also been developed, and in some cases, validated for local conditions or commercialized. The specificity and sensitivity of some ELISAs have been published. The sensitivity can differ between vaccinated and nonvaccinated animals, as well as between different categories of infected animals (e.g., subclinically infected compared to symptomatic animals, or carriers compared to transiently infected animals).

False positives can occur in ELISAs, and these tests are usually used in conjunction with a confirmatory test that has high specificity, such as the EITB. The EITB detects antibodies to the NSP proteins 3A, 3B, 2C, 3D and 3ABC. This test has not been globally commercialized, making it difficult to use and evaluate. It has, nevertheless, been used successfully in South American vaccination campaigns, in conjunction with a 3ABC ELISA, to demonstrate freedom from infection. Retesting positive samples, using combinations of ELISA tests to increase specificity, has been described as a possible alternative to this method.

NSP tests must be validated for each species, and this has been limited by the availability of panels of sera from vaccinated and challenged animals. Different tests have different levels of validation, and they have been validated mainly in cattle. Published information on their validation in other species is incomplete or absent, at present; however, the OIE reports that such validation is ongoing. .

Because antibodies to FMDV proteins can persist after an animal has eliminated the virus, a positive reaction in a serological test does not necessarily mean that the animal is currently infected or a carrier. Carrier animals can be identified by recovering FMDV from esophageal-pharyngeal fluids, using virus isolation or RT-PCR. The ability to identify carriers is influenced by the amount of virus present (which decreases with time), the skill of the operator and other factors. A single probang sample may identify only half of all carriers, but the success rate is improved if testing is repeated at intervals of two weeks. RT-PCR assays are more sensitive than virus isolation, but it is still uncertain whether recovering only RNA should be interpreted as evidence for persistent infections.

The OIE Terrestrial Animal Health Code does not mandate a specific sampling strategy or design prevalence for FMD serosurveillance; it permits the infected country's national authority to choose a method of substantiating freedom from infection, provided the chosen strategy can be justified. Factors that can influence the confidence with which freedom from FMD can be substantiated, using serology, include the sensitivity and specificity of the test system, the prevalence of infection, the characteristics of the population, the herd size and sample size, the herd-based or population-based level of confidence that is used in the design, and the sampling strategy. Due to the limitations of diagnostic tests and the impracticality of testing every animal in the country, surveillance can never entirely guarantee that the country is free of the infection, whether or not vaccination was conducted.

NSP tests must be used for serosurveillance in vaccinated populations. Epidemiological evidence, serological testing, virological testing and the use of sentinel animals can be part of the strategy to determine that virus is not continuing to circulate. Culling herds with reactors, without a follow-up investigation of those reactors, automatically classifies the herd as infected, according to the OIE Terrestrial Animal Health Code.

Designing a sampling strategy with an epidemiologically appropriate design prevalence is a complex task. There is still relatively little information on the probable prevalence of infected animals in a vaccinated herd (particularly subclinically infected animals in emergency vaccinated herds) or on the sensitivity of NSP tests in detecting infected herds. Competent and experienced professionals, as well as

the OIE Terrestrial Animal Health Code, should be consulted when designing a strategy. Targeted surveillance may be valuable when the prevalence of infection is low.

If surveillance misses an infected herd that has one or more carriers, and movement restrictions are lifted, a vaccination to- live policy might result in carriers contacting nonvaccinated animals. The level of risk for virus transmission in this scenario is estimated to be quite low, though still uncertain, in herds or flocks of domesticated ruminants.

5.1 Detecting Acutely Infected Animals and Carriers by Virus Isolation and RT-PCR

Acutely infected animals can be identified using ELISAs to detect viral antigens directly in tissues, as well as by virus isolation or RT-PCR [16]. Lateral flow devices are also commercially available, but have not yet been evaluated by the OIE [16]. In acute disease, the preferred samples according to the OIE are epithelium from unruptured or freshly ruptured vesicles, or vesicular fluid. If vesicles are not available, blood (serum) and esophageal–pharyngeal fluid samples (or throat swabs from pigs) can be collected. FMDV can also be found in oral and nasal swabs, as well as in milk and other secretions and excretions (e.g., saliva, urine or feces), or in samples of myocardial tissues and other organs in fatal cases [16;148]. Oral fluid samples, in particular, have been investigated for the detection of acute infections (< 10 days after inoculation) in cattle and pigs by RT-PCR [149]. Additional technologies such as DNA microarray analysis, rapid pen-side diagnostic techniques (e.g., reverse transcription loop-mediated isothermal amplification) and other assays have also been investigated for FMD diagnosis, and might be useful in future outbreaks [7;150].

Carrier animals can be definitively identified by recovering FMDV from esophageal-pharyngeal fluids, using virus isolation [16;42]. The most suitable samples are taken with a probang cup, which collects mucus and superficial cellular material from the pharynx. The amount of virus varies with time [42]. Recovery is also influenced by the handling of the sample, and the skill of the person recovering the virus [42]. A single probang sample may identify only half of all carriers, but the success rate is improved if testing is repeated at intervals of two weeks. RT-PCR assays can also be used, and may be more sensitive; however, there can be false negatives from nonspecific inhibitors. It is still uncertain whether recovering only RNA and not live virus should be interpreted as evidence for persistent infections [68]. RT-PCR may detect fragments of the viral genome that are not part of a viable virus, and might be positive in animals that have already cleared the infection [42;113]. If possible, both tests should be used together. Kitching found that, when both virus isolation and RT-PCR were employed on the same samples, FMDV could be detected by only one of the two techniques in some cases [42]. In a recent study, real–time quantitative RT-PCR was reported to detect a high percentage of carriers among experimentally infected cattle, during the first 100 days after infection [107].

5.2 Detecting Carriers and Infected Animals by Serological Assays

5.2.1 FMDV Proteins

The FMDV particle consists of a positive sense, single stranded RNA genome inside an icosahedral capsid. The capsid is composed of four proteins, 1A, 1B, 1C and 1D, which are also known as VP4, VP2, VP3 and VP1, respectively [151]. Replication takes place in the cytoplasm. Once the virus enters the cell, the viral RNA is translated into a polyprotein, which is cleaved by viral and host proteinases into more than a dozen proteins. Four of these are the capsid (structural) proteins. The remaining proteins, which are involved in virus replication and various interactions with the host cell, are called the non-structural proteins (NSPs) or non-capsid proteins (NCPs). They include Lpro, 2A, 2B, 2C, 3A, 3B, 3C and 3D, as well as some precursor polypeptides (e.g., 3AB, 3ABC). The 3D protein, which is also called Virus Infection Associated Antigen (VIAA), is a viral RNA-dependent RNA polymerase [42;151]. This protein is often incorporated into the capsid and cannot be purified from conventional inactivated vaccines [151;152]. Antibodies to 3D have been detected in a small number of FMD-naive cattle [153]. Protein 3B

(Vpg), which is necessary for the replication of the viral RNA [151], is also incorporated into the capsid, and cannot be totally be removed [154]. The protease 3C is required for capsid assembly, and is used in the generation of empty capsids in adenovirus-vectored FMD vaccines [151;155]. Lpro and 2A are also proteases involved in cleaving the viral polyprotein, but they are not necessary for capsid assembly [10;151;155]. In addition, Lpro and 3C cleave specific host proteins [10;151]. The remaining NSPs have various roles in replication of the viral genome or host cell interactions [7;151;156].

5.2.2 Seroconversion to Structural and Non-Structural Proteins in Infected and Vaccinated Animals, and DIVA Tests

Infected animals develop antibodies to both structural (capsid) proteins and NSPs. Titers are influenced by the level of exposure to the specific protein. Animals are exposed to NSPs when infected cells are lysed [151], and titers to these proteins seem to be correlated with the extent of virus replication [68]. Titers to NSP proteins may be transient and difficult to detect in some subclinically infected animals, including vaccinated and nonvaccinated animals with low levels of virus replication [42;143;151;157].

Seroconversion to structural proteins (SPs) occurs earlier than to NSPs, and the titers are usually higher [130;153]. In cattle, antibodies to SPs have been detected as soon as 3–4 days after infection [151], while antibodies to the NSP proteins 3A, 3B, 3D, 3AB and 3ABC have been found as early as 7–10 days [151;158-160]. In one study, all cattle had titers to structural proteins on day 8, and developed antibodies to NSPs beginning on days 8-10 [159]. In the same experiment, antibodies to SPs could be found 8-14 days after infection in sheep. Titers to some NSPs were first detected on day 10 in two sheep, but two other animals did not respond to these proteins until day 14 or 22. In sheep, responses to the 3D protein occurred later than responses to 3ABC and 3AB. Similarly, another study found that antibodies to NSPs appeared during the first week after infection in some sheep, but not until the second week in most, and the third week or later in a few animals [98]. Some field studies in goats have found greater levels of seroconversion to NSPs than SPs; however, it is possible that test sensitivity differs in this species, or that nonspecific cross-reactivity occurs in the serum of some goats [79;100;161]. In pigs, Chen et al. first detected antibodies to NSPs 6 to 8 days after infection [162]. Other reports also suggest that these antibodies can be recognized in pigs within the first 1-2 weeks [151]. One study detected NSP titers in water buffalo after 9 to 19 days [82]. Titers to structural proteins may persist for the life of the animal, but antibodies to NSPs decline and become undetectable sooner [151]. The immune responses to 3ABC and 3AB appear to persist longer than antibodies to other NSPs, with detectable titers to 3ABC reported for 1-3.5 years in some studies [151;160]. Titers to the NSP protein 3B have been reported to persist for up to 364 and 301 days in cattle and swine, respectively, while antibodies to 2C were found in some cattle for up to a year [42;130;151].

Vaccination primarily induces antibodies to structural proteins [151]. With a sufficiently purified vaccine, vaccinated animals will be exposed to most NSPs only if they become infected with a field virus. For this reason, tests that detect titers to NSPs can be used to differentiate vaccinated from infected animals (DIVA tests). However, insufficiently purified vaccines can contain low levels of NSPs, and may induce titers to these proteins. Vaccine purity is especially important when animals must be vaccinated multiple times [42]. Because vaccination can reduce virus replication, titers of antibodies to NSPs tend to be lower in vaccinated than nonvaccinated animals, and seroconversion can be delayed or even absent [130;143;151;163-165].

5.2.3 Uses of Serological Tests in Outbreaks

In FMD outbreaks, serological tests can be used to confirm suspected cases, monitor the efficacy of vaccination, and provide evidence for the absence of infection. Test validation must consider the purpose of the assay [16]. For instance, test cut-offs may be set at a different level when the test is intended to certify that individual animals are uninfected than when it is used for herd-based serosurveillance. Test cut-offs may also be influenced by the epidemiological situation. In South America, there was a higher

background in NSP tests when vaccination programs had been conducted in the area for at least the previous 5 years, compared to areas that did not vaccinate [130].

5.2.4 Serological Tests that Detect Antibodies to Structural Proteins

Serological tests based on structural proteins are serotype-specific [16;70]. They are highly sensitive if closely matched to the field virus. Their disadvantage is that a single assay cannot be used to detect antibodies to field viruses of different serotypes, or to screen for infections with viruses of unknown serotype. In addition, these tests cannot distinguish whether antibodies to structural proteins were stimulated by vaccination or infection. For this reason, they are useful for detecting infections only in nonvaccinated populations. SP tests may also be employed to monitor vaccine titers. In the OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals, some recommended serological SP assays (and the prescribed tests for trade) include the virus neutralization test (VNT), the solid-phase competition ELISA (SPCE) and the liquid phase blocking ELISA (LPBE) [16]. VNT, which uses live virus and requires cell culture facilities, takes 2–3 days to complete. ELISAs are faster than VNT, and do not require live virus or culture facilities. Screening with an ELISA and confirming positive reactions with VNT minimizes the occurrence of false-positives.

5.2.5 Serological Tests that Detect Antibodies to NSPs

Tests that detect antibodies to NSPs can identify infections in either vaccinated or nonvaccinated animals [16]. Because NSPs are conserved across serotypes and strains, a single assay can recognize infections with all FMD viruses [16]. However, it should be noted that the Lpro and 3C NSPs of SAT viruses from southern Africa may differ from these proteins in A, O and C and SAT viruses from eastern Africa [151]. It might be possible for this difference to influence the detection of some SAT viruses in NSP assays based on these proteins. Tests that detect antibodies to NSPs are less sensitive than tests based on structural proteins, and may not detect animals with limited virus replication [42;130;143;151;157; 163-165].

In the OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals, the recommended NSP assays are ELISAs and immunoblotting (e.g., the enzyme-linked immuno-electrotransfer blot [EITB]) [16]. In cattle, 3ABC and 3AB are reported to be the most reliable markers to distinguish vaccinated animals from those infected by field strains [16;153;158;166;167]. A number of commercial 3ABC ELISA kits, as well as some "in house" tests, are available [153]. A blocking 3AB ELISA was used to detect infected pigs during a vaccination campaign in Taiwan in 1997, and in-house 3AB tests have also been used in other countries [153;157;160;167;168]. ELISAs based on additional NSPs have been described, and in some cases, validated for local conditions [167-172]. At least one of these tests, a 3B assay, has been commercialized. Some NSP test formats use species-specific conjugated antibodies, and test kits are likely to be available for only a limited number of animal species [172]. Other ELISAs (e.g., competitive ELISAs) can be used in multiple species [172], provided they have been validated for each.

The specificity and sensitivity of some ELISAs have been published [153;154;164;165;172-174]. In general, the studies demonstrate that the specificity of these tests is high for sera from both vaccinated and nonvaccinated animals, but the sensitivity is higher for nonvaccinated than vaccinated animals [164;165;172-174]. Only a handful of studies have compared assays directly, under the same conditions. In one study, the estimated specificity for five 3ABC ELISAs and a 3B test in vaccinated and nonvaccinated cattle ranged from 97% to 98.5%, and improved to 98-100% when samples were retested using the same assay [173]. In nonvaccinated cattle, the sensitivity was 100% for all tests when the animals had been exposed less than 28 days previously, and 92% to 100% if they were tested 28-100 days after infection, which is the most critical period for post-outbreak serosurveillance. During this same period, test sensitivities varied from 53% to 75% in all vaccinated cattle with evidence of infection (i.e., clinical signs, virus isolation or increased antibody titers to structural proteins), and from 68% to 94% in carriers. The three tests that performed best in detecting carriers, with sensitivities of 86% to 94%, were the NCPanaftosa from PANAFTOSA, 3ABC trapping-ELISA from IZS-Brescia and Ceditest® FMDV-

NS from Cedi Diagnostics (now PrioCHECK®, Prionics B.V. Lelystad, The Netherlands). In this study, all 6 ELISAs had very poor sensitivity (15% to 27%) in animals that had been infected but had never been carriers; however, these animals would not be at risk for transmitting virus after they recovered. Using field samples collected during post-outbreak surveillance in Israel and Zimbabwe, the sensitivities of the same 6 ELISAs ranged from 72% to 100%, and the same three tests had the highest sensitivity (97% to 100%) [173]. Another study reported similar results. Engel et al. reported specificities of 96% to 99% for five 3ABC ELISAs and a 3B ELISA in vaccinated and non-vaccinated cattle [165]. In nonvaccinated cattle, the tests all had estimated sensitivities of 95% to 97%, with no significant differences between them; however, in vaccinated cattle, which are likely to have lower titers, the sensitivity ranged from 57% to 94%. A third study, which used a panel of 36 bovine sera to evaluate an in-house test and 4 commercial ELISAs, confirmed that subclinically infected cattle were more difficult to detect than symptomatic animals [164]. In the latter study, 3 ELISAs were less sensitive when more time had elapsed since infection, but the Ceditest FMDV-NS (PrioCHECK) and an in-house test (Istituto Zooprofilattico Sperimentale, Brescia) were unaffected [164]. Only one of the studies examined NSP ELISAs for use with sheep and pigs, and only limited numbers of serum samples were available [173]. In sheep, the specificity of four ELISAs was reported to be 98% to 100% [173]. Their sensitivity was 100% in nonvaccinated animals, and 33% to 67% in vaccinated animals. In pigs, the specificity of five ELISAs ranged from 97% to 100%, with sensitivity of 100% in nonvaccinated animals and 44% to 69% in vaccinated animals [173].

False positives can occur in ELISAs, and these tests are usually used in conjunction with a confirmatory test that has high specificity, such as the EITB [16;130;151;155;162]. Retesting positive samples, using combinations of ELISA tests to increase specificity, has also been described [131;173]. This approach was used during the 2009 outbreak in Taiwan to increase the positive predictive power of the tests, at a time when the incidence of infection was very low [175]. However, there remain limitations in the sensitivity and specificity of NSP tests, which are not easily overcome by combining them [176].

The EITB uses immunoblotting (western blotting) to detect antibodies to 3A, 3B, 2C, 3D and 3ABC [16]. The sample is considered to be positive if antigens 3ABC, 3A, 3B and 3D (±2C) are positive, and negative if two or more antigens have densities less than control sera [16]. If neither case applies, then the interpretation is indeterminate. This test has been provided by a central reference laboratory in South America to national laboratories on that continent [153]. It has not been globally commercialized, making it difficult to use and evaluate [177]. It has, nevertheless, been used successfully in South American vaccination campaigns, in conjunction with a 3ABC ELISA, to demonstrate freedom from infection. The diagnostic specificity of the South American ELISA/ EITB system is reported to be greater than 99% in animals vaccinated once or multiple times [130]. In the field, a small number of false negative and false positive results would be expected. As of 2005, no false negatives had been detected among known carriers in South American eradication programs [130].

5.2.6 The Use of NSP Tests to Detect Infected Herds

Because they allow FMDV infections to be recognized in vaccinated herds, NSP tests have made vaccination-to-live a possibility. Although these tests have limitations in identifying individual animals, they are valuable as herd tests, and can be used as part of the procedure to regain FMD free status [42;70;130;151;153;154;178;179]. In addition to detecting ruminant herds with carriers, they could be used to detect virus circulation in large herds of swine (e.g., if FMDV is being maintained by passage from pig to pig) [180].

The OIE Terrestrial Animal Health Code does not mandate a specific sampling strategy or design prevalence for FMD serosurveillance; it permits the infected country's national authority to choose a method of substantiating freedom from infection, provided the chosen strategy can be justified [70]. Particular difficulties in conducting surveillance in vaccinated populations include the low prevalence of

infection overall, low prevalence of infected animals in an infected herd, increased probability of false positive tests, and the tendency for infections to cluster between and within herds [181]. Some of the factors that can influence the confidence with which freedom from FMD can be substantiated, using serology, include the sensitivity and specificity of the test system, the prevalence of infection, the characteristics of the population, the herd size and sample size, the herd-based or population-based level of confidence that is used in the design, and the sampling strategy [131]. Due to the limitations of diagnostic tests and the impracticality of testing every animal in the country, surveillance can never entirely guarantee that the country is free of the infection, whether or not vaccination was conducted [131;181].

NSP tests must be used for serosurveillance in vaccinated populations. Because antibodies to these proteins can persist after an animal has eliminated the virus, a positive reaction in a serological test does not necessarily mean that the animal is currently infected or a carrier [130;151]. Serological tests used for initial screening are also chosen for their sensitivity, and will falsely identify a certain number of uninfected animals as infected (false positives). If a herd NSP test is positive, a decision must be made either to slaughter the entire herd because it might contain carrier animals, or to conduct more tests to evaluate whether the virus is still present and continuing to circulate [180]. No method to detect carriers is completely reliable, and testing is labor intensive and expensive. However, culling entire herds may result in the elimination of very large numbers of animals in the U.S. In addition, culling herds without a follow-up investigation of reactors automatically classifies the herd as infected, according to the OIE Terrestrial Animal Health Code [70;131].

The OIE-recommended investigation of herds with seropositive animals includes the use of clinical signs, epidemiological studies and supplementary laboratory tests including serology and, where possible, virological tests [70]. Confirmatory serological tests should have high specificity to reduce false positives, and their sensitivity should approach that of the screening test. The EITB or another OIE-accepted test is recommended [16;70]. Epidemiological evidence is used to exclude the possibility that the animal is seropositive because the virus is circulating. A suggested strategy is to collect a second (paired) serum sample from the animals in the original herd test [70]. If the virus is not circulating, the number of animals with antibodies to NSPs in the population should be (statistically) equal to or lower than the number of seropositive animals in the first test. If the animals that were originally tested are not accessible or individually identified, or if they have been vaccinated since the first sample was taken, then a new serological survey of the premises, with paired samples taken from individually identified animals, should be done. In addition, epidemiological studies with serological assays are carried out in contact animals. Clustering of positive animals is suspicious, whereas a low number of seropositive, unclustered animals at levels below the expected false positive rate could occur without FMDV being present [176]. The OIE Terrestrial Animal Health Code notes that sentinel animals of the same species (young, nonvaccinated animals or animals with no maternal antibodies) can be tested by serological assays [70]. If nonvaccinated, susceptible animals of other species are in contact, they can also be used as sentinels for additional evidence that the virus is not circulating. Sentinel animals may be tested for antibodies to either SPs or NSPs. Some sources have suggested that sentinel animals may be of limited value in vaccinated herds due to the low rate of transmission [179]. The OIE Terrestrial Animal Health Code states that a reactor in the initial serological screening may be classified as negative if all follow-up tests indicate that there is no evidence the virus is present [70]. If follow-up testing is not done, or if any tests suggest the virus has not been eliminated, the animal is classified as FMD positive.

Designing a sampling strategy with an epidemiologically appropriate design prevalence is a complex task, and the OIE recommends consulting with competent and experienced professionals in this field to generate a justifiable strategy [70]. Some general principles of surveillance for FMD and the establishment of a sound, science-based method for substantiating FMD-free status have been published [181] They include a discussion of the importance of targeted surveillance in high risk groups, when the

population has high levels of immunity (e.g., from vaccination) that could mask and/or limit virus circulation.

There are still uncertainties in developing strategies to detect infected herds. Establishing a sampling rate that can detect at least one previously infected animal in a vaccinated herd with the necessary statistical certainty may be difficult. In South American vaccination campaigns, a 3ABC ELISA has been used for initial screening, followed by the EITB [130]. Combinations of ELISA NSP tests have been described that would allow a 5% prevalence of carriers to be detected at 95% confidence [131]. Some combinations required that the herd contain at least 30 cattle, while others necessitated that it have more than 50 cattle [131]. There is some uncertainty that NSP testing can reliably determine that small herds are free from infection (the "small herd problem"), because there may be insufficient numbers of epidemiologically linked animals to detect the design prevalence at the required statistical power ([182] cited in [155]; and [131;183]). For example, if the test used has 80% sensitivity, at least two infected animals in the herd must be sampled for 95% confidence that one of these animals will be detected [131]. Paton et al. reported that a single infected animal cannot be detected at 95% confidence, if the herd contains fewer than 30 animals and the test has a sensitivity of 80%, even if the test specificity is 100% and all of the animals are sampled [131]. They suggested that difficulties in detecting infected animals in small herds might be solved by not vaccinating small herds or using only vaccination-to kill in these herds, or by requiring additional biosecurity restrictions for vaccinated small herds after the outbreak. Small herds may present only a low risk for virus transmission, and vaccination of these herds might not be a priority [131]. Another possibility would be to test greater numbers of small herds than required to demonstrate that the proportion of infected herds is less than 2% [131]. A 2008 modeling study from Arnold et al., however, suggests that the small herd problem may not exist [119]. This study reports that the number of carriers after emergency vaccination may not depend on the size of the herd. For this reason, carriers might actually be easier to detect in small herds (at a 5% prevalence and 95% level of confidence) because all of the animals are sampled. However, this study also casts doubt on a herd-based approach to sampling (see below) because of the very low number of carriers expected in each herd. In the E.U., the European Directive on FMD Control mandates that all vaccinated ruminants and their nonvaccinated offspring be sampled [176;179]. This approach aids in preventing any small herd problem. Sampling all vaccinated animals in large herds of animals (e.g., herds of pigs, or large ruminant herds in the U.S.) may be difficult or impractical [179]. It might be possible to use somewhat different surveillance strategies or sample numbers in pigs than ruminants, as pigs are not thought to become carriers, circulating viruses tend to become clinically apparent even in vaccinated herds of swine, and NSP titers may be relatively short-lived in this species [176].

There is still relatively little information on the probable prevalence of infected animals in a vaccinated herd (particularly subclinically infected animals in emergency vaccinated herds) or on the sensitivity of NSP tests in detecting infected herds [131;180]. Two recent modeling studies suggest that the expected prevalence of carriers after emergency vaccination may be very low, and serological detection of herds with carriers may be difficult [119;184]. It should be noted that both studies are based on information from the 2001 outbreak in the U.K., and may not be applicable to the U.S. The study by Schley et al. reported that fewer than 2.5 carriers would be expected on randomly selected U.K. farms, and test sensitivity would need to be high for detection [184]. Arnold et al. found that the expected prevalence of carrier animals after emergency vaccination is approximately 0.2%, and herds may contain only one or two carriers on average [119]. When more animals are infected in the herd and more carriers might be expected, the herd is likely to be identified by clinical signs and the animals slaughtered during the outbreak. These authors concluded that, because the number of expected carriers is so low, a herd-based approach and a 95% level of confidence will be unable to detect many infected herds that contain carriers [119]. They suggest that consideration be given to testing all animals in a herd and removing only those that test positive. The removal of reactors, as opposed to culling of the entire herd, would allow the use of tests that have high sensitivity with decreased emphasis on high specificity. Some other sources have also

suggested the possibility of removing seropositive animals from herds, followed by re-testing and follow-up investigations to confirm freedom from infection in herds that had reactors [131]. This approach would need to be justifiable to the OIE, in order to substantiate freedom from infection. The limitations of NSP assays in detecting individual animals could be an issue [179].

If surveillance misses an infected herd that has one or more carriers, and movement restrictions are lifted, a vaccination to- live policy might result in carriers contacting nonvaccinated animals. The level of risk for virus transmission in this scenario is estimated to be quite low, though still uncertain, in herds or flocks of domesticated ruminants [14;42;84]. If viruses continue to circulate in vaccinated populations, the evolution of new variants might favor the development of vaccine resistance [185-189]. Whether the presence of carriers alone can promote the evolution of FMDV strains is still unclear [10].

5.2.7 Validation of NSP Tests

NSP tests must be validated for each species, and this has been limited by the availability of panels of sera, especially from vaccinated and challenged animals [178]. Different tests have different levels of validation, but they have been validated mainly in cattle [42;70;151;153;154]. Only limited information has been published for other species [153;154].

Most studies in sheep have examined only small numbers of animals, and are inadequate to make conclusions about the sensitivity and specificity of the various NSP tests in this species [154]. Some 3ABC tests have been able to detect infections among both vaccinated and nonvaccinated sheep [49;100;138;143;190]. As in cattle, it is more difficult to detect infected animals when virus replication is low. Parida et al. found that a 3ABC ELISA had good sensitivity for detecting both heavy virus shedders and carriers among experimentally infected sheep; however, it was not very sensitive in detecting animals with subclinical infections or low levels of replication [143]. Likewise, Brocchi et al. reported sensitivities of 100% for four NSP ELISAs in nonvaccinated sheep, but only 33% to 67% in vaccinated sheep [173]. Test specificities did not differ between vaccinated and nonvaccinated sheep in this study, and ranged from 98% to 100%.

A few comparative studies have examined NSP ELISAs in pigs. In experimentally infected pigs tested with three commercial ELISA tests, seroconversion to NSP proteins correlated with the severity of clinical signs and amount of virus replication [113]. No single NSP test detected all infected pigs, but by combining tests, sensitivity and specificity could be increased [113]. Another study, which compared the same three tests, concluded that the 3ABC Ceditest (PrioCHECK) NSP ELISA had the best profile, based on the highest sensitivity and specificity, and the least reactivity with residual NSPs in vaccinated pigs [162]. Brocchi et al. reported that five 3ABC NSP ELISAs had sensitivities of 100% in nonvaccinated pigs, but only 44% to 69% in vaccinated pigs [173]. Test specificities did not differ between vaccinated and nonvaccinated pigs in this study, and ranged from 97% to 100%. There is currently no information about the use of NSP tests in ranched cervids or wildlife [154].

Additional information on the sensitivity and specificity of various tests is available from some publications and/or manufacturers [153;154]. It is difficult or impossible to compare the sensitivity and specificity of different tests unless they are evaluated under the same conditions, e.g., in a single study [153;154]. The formulations of test kits also change frequently [153]. However, a recent review notes that commercial NSP tests are generally comparable in performance and adequate for use as herd tests [154]. Validation of a test may be necessary or desirable by the country using the test, with adjustment of test cutoffs according to the situation [153]. For example, adjustments could increase sensitivity with the tradeoff of reduced specificity [153;154].

5.2.8 Serological Assays in Development

Several multiplex tests, based on reactivity to more than one NSP, have been described in the literature [154]. Some of these tests include a dot immunoblot assay similar to the EITB, ELISAs and liquid array

tests [10;154;191;192;192-195]. These assays provide information about the relative responses to multiple FMDV protein signatures (e.g., 3A, 3B, 3D and 3ABC, in one liquid array test in development in the U.S.) [10;192;194;195]

Serological tests to detect the NSP protein 3D can be used in nonvaccinated animals, or with recombinant vaccines that do not contain this protein, such as an hAd5-vectored FMD vaccine (described below) [10]. A 3D liquid phase blocking ELISA has for cattle and pigs has been investigated [10]. Antibodies to 3D can also be detected with a traditional agarose gel immuno-diffusion (AGID) test, which has a long turnaround time [10]. 3D tests are not useful in animals vaccinated with conventional inactivated vaccines, which always contain this protein even when they are purified [151;152].

FMD-specific IgA, which occurs in the saliva of recovered or vaccinated cattle, might be useful in the detection of carriers [42]. The levels of these antibodies tend to be higher in carriers than in animals that have cleared the virus, probably because their production continues to be stimulated locally by FMD antigens. ELISAs that quantify the level of specific IgA in saliva have been developed, and might eventually be useful as a herd test [42]. However, the levels of specific IgA are not elevated in some individual carriers and this system still requires development. Unpublished work with one IgA ELISA suggested it had promising sensitivity and specificity ([196] cited in [154]).

6. FMD VACCINES

Summary

Nearly all currently licensed FMD vaccines are killed vaccines containing chemically inactivated virus. Conventional (standard potency) vaccines are still used routinely to control FMD in endemic areas. They usually contain lower doses of antigen and are less potent than emergency vaccines.

Aluminum hydroxide adjuvanted FMD vaccines are effective in cattle, sheep and goats, but function poorly in pigs, while oil-adjuvanted vaccines can be used in any species. FMD vaccines with oil adjuvants are at least as effective as those containing aluminum hydroxide. The shelf life of conventional, fully formulated FMD vaccines is usually 1–2 years at 4°C.

Purified vaccines should be used in programs where infections with the field virus must be identifiable in vaccinated animals. If less purified vaccines are used, vaccinated animals may develop low titers of antibodies to NSPs, which are the basis for DIVA tests.

Non-commercial FMD vaccine banks, which can be activated in emergencies, are maintained in some individual countries. There are also two multinational cooperative banks: the North American Vaccine Bank (NAFMDVB) for the United States, Canada and Mexico, and the European Union Vaccine Bank (EUVB) for the E.U. Noncommercial vaccine banks usually operate on a relatively small scale, and an individual bank may be able to meet only the initial needs during an outbreak. Because some stocks are duplicated in different banks, it might be possible to obtain additional vaccine supplies from other countries. In 2006, representatives of FMD vaccine banks approved the creation of an international FMD vaccine bank network, to operate under the auspices of the OIE. Some of the goals of the network include addressing sudden increases in the demand for vaccine and establishing a global vaccine reserve for FMD, as well as harmonizing vaccine and test standardization and certification.

FMD vaccine banks usually store concentrated antigens, which can be kept at ultra-low temperatures for many years. In an outbreak, banks can rapidly formulate stored antigens into complete vaccines. These vaccines can be tailored to the epidemiology of the outbreak. Banks are usually able to make either monovalent or polyvalent vaccines that contain oil or aluminum hydroxide/ saponin as the adjuvant. It is possible to adjust the potency of the vaccine according to need and to the relatedness of the field and vaccine strains. The time between receipt of the order and vaccine delivery has been estimated to be 4 to

13 days, depending on the distance the antigens and/or vaccine must be shipped, the daily finishing and filling capacity of the manufacturer and the availability of flights. At an international conference for representatives of vaccine banks, manufacturers' estimates for vaccine formulation were 3-7 days, with the period between ordering and application in the field likely to be at least 6-10 days. Normal batch or serial tests to demonstrate purity, safety and potency would take additional time, if these tests must be done for licensing.

Vaccine banks can store only a limited number of serotypes and strains of FMDV. Vaccine strains held in banks are generally those felt to have the greatest risk of introduction, based on the worldwide epidemiological situation. These stocks are under continual review. The integrity of the antigens must be maintained while they are frozen, stored, thawed and diluted.

The NAFMDVB on Plum Island contains stockpiled vaccine antigen concentrates. When these antigens are needed, they must be shipped to the country that produced the antigen, and formulated and finished by the antigen manufacturer. Vaccines manufactured in foreign countries that meet efficacy, potency, purity and safety standards could also be stored in the NAFMDVB, or stored overseas and made available through "just in time" supply contracts, if the manufacturing methods and production facilities are approved.

Vaccines may be licensed and distributed with a full product license, or they may receive a conditional biologics license for use in specific conditions, e.g., if the product will be used by or under the supervision of the USDA in an emergency animal disease outbreak. The USDA has mechanisms for expedited product approval, and if necessary, can exempt products from some of the regulatory requirements for full product approval during emergencies.

Commercially available, conventional FMD vaccines can be an alternative to emergency vaccines in an outbreak. Commercial manufacturers have larger operations than noncommercial vaccine banks, and regularly produce these vaccines for countries where FMD is still endemic. A disadvantage is that conventional FMD vaccines typically contain lower doses of antigen and are less potent than emergency vaccines. A commercial vaccine manufacturer might also be unavailable if it is already contracted to produce vaccines for other customers. One quadrivalent FMD vaccine has been permitted for distribution and sale in the U.S. in the event of an outbreak. At present, the manufacturer manufactures sufficient vaccine only to meet the needs of its current customers.

If a new vaccine must be prepared from an outbreak strain, the field virus must first be adapted to culture. An experimental approach, which might bypass this step, involves the development of new vaccine strains by modifying cDNA clones of existing strains. Once a field virus has been adapted to grow in culture, the lead time for vaccine preparation is 1 to 6 months.

A human adenovirus 5-vectored serotype A_{24} FMD vaccines has received a conditional license in the U.S., but is not being manufactured at this time. This vaccine can be produced without the need for high biosecurity conditions, and is compatible with DIVA testing. It is made as a ready-to-use vaccine, and initial estimates suggest that it can be stored frozen for at least 3 years. Additional serotypes and strains of hAd5-vectored vaccines are in development.

Several additional approaches to experimental FMD vaccines, including other viral-vectored vaccines, DNA vaccines, virus-like particles, and subunit or peptide vaccines are under investigation. A leaderless vaccine construct is being developed as a safer platform for manufacturing inactivated FMD vaccines.

6.1 Types of FMD Vaccines

Nearly all fully licensed, commercially produced FMD vaccines are inactivated (killed) vaccines containing chemically inactivated virus [151]. Similar vaccines have been manufactured since the 1950s, and have been used successfully in a number of control or eradication programs. Both monovalent and multivalent FMD vaccines are produced. One serotype A, adenovirus-vectored vaccine has received a conditional license in the U.S. [197], but is not commercially produced at present. China licensed a synthesized peptide, type O, FMD vaccine in 2007. Many currently available vaccines for FMD produced around the world can be found at the following website: http://www.cfsph.iastate.edu/Vaccines/index.php?lang=en. A number of experimental approaches are also under investigation.

Conventional live attenuated vaccines are unacceptable for FMD [16]. When attempts were made to produce such vaccines in nonsusceptible hosts, the attenuated viruses tended to revert and become virulent [155;198]. Live attenuated vaccines would also be undesirable in that they would not allow infections to be recognized in vaccinated animals, and there would be a risk of shedding the vaccine virus [16].

6.2 Vaccine Licensing

The Center for Veterinary Biologics in the USDA, the USDA's National Veterinary Stockpile (NVS), and other agencies may be involved in purchasing vaccine antigen concentrates and/or finished routine or emergency use vaccines [199]. NVS may also contract with manufacturers for immediate access to existing stocks of licensed emergency use vaccines. Vaccines may be licensed and distributed with a full product license, or they may receive a conditional biologics license for use in specific conditions, e.g., if the product will be used by or under the supervision of the USDA in an emergency animal disease outbreak [199].

For a vaccine to be given a full product license, the manufacturer must conduct extensive efficacy, purity and safety testing [199-201]. Steps in the licensing of vaccines in the U.S. include a review of the data from the manufacturer to support the product and label claims; inspections of manufacturing processes and practices; confirmatory testing of the biological seeds, cells and product; post-licensing monitoring including inspections and random product testing; and post-marketing surveillance of product performance [199]. In standard licensing, the seed materials, product ingredients and final product must be completely characterized and tested for purity. Safety and efficacy tests must also be done, and product stability and duration of immunity (DOI) must be evaluated. All of these steps may not be possible during an animal disease emergency. The USDA has mechanisms for expedited product approval, and can exempt products from some of the regulatory requirements for full product approval during emergencies [199]. However, every attempt is made by the CVB to establish a reasonable expectation of purity, safety, potency and efficacy prior to the use of any vaccine. In addition to potential harm to animal, human and environmental health, the risk of lawsuits if problems occur must be considered [109;199].

6.3 Vaccines Manufactured Using Live Virus

6.3.1 Inactivated FMD Vaccines

Inactivated FMD vaccines are classified into two broad categories, conventional vaccines and emergency vaccines. Conventional (standard potency) vaccines are still used routinely as a prophylactic measure for controlling FMD in endemic areas. They usually contain lower doses of antigen and are less potent than emergency vaccines. Both aluminum hydroxide and oil adjuvanted FMD vaccines are produced. Aluminum hydroxide/ saponin adjuvanted FMD vaccines are effective in cattle, sheep and goats, but function poorly in pigs, while oil-adjuvanted vaccines can be used in any species [1;10;68;109]. Improved antibody responses and potency have been reported for double oil emulsion compared to water-in-oil single emulsion vaccines [10;202-204]. Oil adjuvanted FMD vaccines are at least as effective as aluminum hydroxide adjuvanted vaccines in ruminants [10;109;205], but whether they induce better

immunity has been debated. Some studies have reported more effective immune responses with oil [206-209], while others found no difference between the two adjuvants ([22;137;207;210;211]; [212;213] cited in [210]). Currently, the OIE/Food and Agriculture Organization (FAO) World Reference Laboratory notes that the use of oil adjuvants is expected to result in better efficacy [8], while the OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals does not indicate a preference of adjuvant in ruminant vaccines [16]. Vaccines with oil adjuvants are simpler to manufacture and are reported to have a better shelf-life [1;68].

Novel adjuvants that may improve efficacy are being investigated, and FMD vaccines containing some of these adjuvants were reported to provide good early protection from challenge [214;215]. At present, there are no sufficiently proven adjuvants that would be suitable replacements for oil or aluminum hydroxide [177].

6.3.2 Production of Inactivated FMD Vaccines

The production of large quantities of FMDV requires high containment BSL-3 (containment Group 4) facilities [10;16]. It is illegal to possess live FMDV on the U.S. mainland, and standard inactivated vaccines cannot be manufactured in the U.S. [163]. However, vaccine antigens made in other countries are stockpiled in the North American FMD Vaccine Bank (NAFMDVB) at Plum Island Animal Disease Center (PIADC). Since 2007, the USDA's Center for Veterinary Biologics (CVB) has also been allowed to consider "Distribution and Sale" permit applications for inactivated FMD vaccines that have been manufactured in foreign countries [10]. Vaccines that meet efficacy, potency, purity and safety standards could be stored in the NAFMDVB, or stored overseas and made available through "just in time" supply contracts, if the manufacturing methods and production facilities are approved [10]. The latter vaccines, if obtained under just in time contracting, would be acquired through the National Veterinary Stockpile and would be a separate U.S. resource; they would not belong to the tripartite NAFMDVB. The Department of Homeland Security (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 [216]. The vaccine is a quadrivalent FMD vaccine (serotypes A_{24} Cruzeiro, A2001 Argentina, C_3 Indaial, and O_1 Campos) produced by Biogenesis Bago in Argentina.

Most modern inactivated FMD vaccines are produced in BHK-21 suspension cell cultures [151]. Older methods include growing the virus in primary bovine tongue epithelial cells (Frenkel method) or in rabbits (lapinized). Formaldehyde was originally employed to inactivate the virus, but this chemical has an exponential inactivation curve, and some vaccine related outbreaks occurred when it was used [151]. Formaldehyde was replaced by aziridines (e.g., ethyleneimine, usually in the form of binary ethyleneimine) in the 1970s [10;16;151]. Time and temperature conditions for inactivation must be validated for the conditions and equipment [16]. 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 [22]. The inactivated antigens can be concentrated by polyethylene glycol precipitation, ultrafiltration, or cycles of adsorption and elution using polyethylene oxide [16;151]. To purify the vaccine, NSPs can be separated from whole virus particles by chromatography or other techniques. Purified vaccines should be used in programs where infections with the field virus must be identifiable in vaccinated animals. As support for a manufacturer's claim that a vaccine does not induce antibodies to NSPs, the OIE suggests vaccinating cattle at least 3 times with the maximum antigen content allowed in that vaccine, and testing them for NSPs 30-60 days after the final dose [16].

Concentrated, purified, tested FMDV antigens can be formulated directly into a complete vaccine, or the antigens can be frozen at ultra-low temperatures (usually in the vapor phase over liquid nitrogen), to be stored until required. Fully formulated vaccines have a relatively short shelf-life, [10;109;152] but vaccine bank antigens frozen at –70°C or lower can be stored for at least 5 years [152] and in some cases,

for more than 15 years [217]. FMD vaccines are formulated by dilution in a suitable buffer, with the addition of adjuvants and other vaccine components [16;19;22]. The final product is tested for safety and potency.

The shelf life of conventional formulated FMD vaccines is usually 1–2 years at 4°C (range 2-8°C) [16]. Some emergency FMD vaccines may be less stable [109]. This effect, which has been reported for some vaccines but not others, might be caused by proteases from the culture harvest and/or the type of formulation [109]. FMD vaccines are considered to be temperature labile, and should not be frozen or stored above a target temperature of 4°C [16]. Preliminary evidence presented at the 2010 European Commission for the Control of FMD meeting suggested that freezing might not adversely affect vaccine potency, and might extend the shelf-life [218]. In cattle immunized with oil-adjuvanted vaccines frozen for 14 months at –20°C, the mean neutralizing antibody titers were 84-90% of the titers after immunization with the same vaccine stored at 2-8°C for one month. In contrast, the same vaccine stored at 4°C for 13 months induced titers that were 36-73% of the titers from the vaccine stored for one month. The same group also evaluated vaccines frozen for 41 months, using serology in guinea pigs, and found that they maintained their potency better than the equivalent vaccine stored at 4°C [218]. Studies performed more than a decade ago suggested that it might be possible to store fully formulated vaccines by a novel procedure with the stratification of individual vaccine components and storage at ultra-low temperature [1]. Early studies suggest this procedure might extend the shelf life of the vaccine to at least 40 months [1]. This technique does not seem to have been investigated further, and both approaches described above are still experimental.

Strain-related differences may affect vaccine manufacture and storage. When used in a vaccine, serotype O is less immunogenic than other serotypes, and requires a higher antigen payload [10;137;219]. SAT-1, SAT-2, and SAT-3 viruses are less stable than other serotypes [10], and SAT-2 and SAT-3 viruses can dissociate under mildly acid conditions [220]. To ensure that vaccines containing the SAT serotypes are potent and remain so during storage, extra quality assurance steps must be taken [10].

6.3.3 Vaccine Banks

Vaccine banks (also known as antigenic banks or strategic reserves) store a variety of FMDV serotypes and strains, which can be used if an outbreak occurs. Banks may contain either ready-to-use vaccines or vaccine antigens that will be formulated, if needed, into complete vaccines. The earliest FMD vaccine banks stockpiled fully formulated, inactivated vaccines; however, these vaccines have a relatively short shelf-life and must be discarded periodically, making such banks expensive [152;201]. Currently, they usually store concentrated antigens, which are kept at ultra-low temperatures. FMD vaccine banks could also be used to stockpile other types of vaccines, such as hAd5-vectored constructs, in a ready-to-use form.

Non-commercial FMD vaccine banks are maintained in some individual countries, either in national institutes or by commercial vaccine producers. There are also two multinational cooperative banks: the North American Vaccine Bank (NAFMDVB) at the PIADC for the United States, Canada and Mexico, and the European Union Vaccine Bank (EUVB), which stores antigens in France and Italy for the E.U. These banks were uncommonly used in the past, but activation has become somewhat more frequent in recent years [109;201]. As of 2015, the NAFMDVB has never been activated. The first activation of the EUVB was for an outbreak in the Balkans in 1996 [109]. The EUVB also supplied vaccines to Japan (which did not use the vaccine) and the Republic of Korea in 2000, to Turkey in 2000 and 2006, and to Iraq in 2009 [109;152;201;221]. The International Vaccine Bank (IVB) (disbanded in 2003) was located in the UK. It was unusual in having its own independent, non-commercial facility to formulate vaccines; other vaccine banks may have contracts with manufacturers to formulate any vaccines needed [201]. The IVB had only one large-scale activation, for the 2001 epizootic in the U.K., and the vaccine was not used [109]. Some vaccine banks in individual countries are relatively active. The Argentinean FMD Vaccine

and Antigen Bank provided more than 187 million doses of vaccine between October 2000 and May 2002, to help control an epizootic in Argentina [222]. It also supplied vaccine to Uruguay [201]. A new regional FMD antigen bank, which would contain strains exotic to the region and could coordinate with the NAFMDVB, has been recommended for South and Central America [8].

Vaccine banks can store only a limited number of serotypes and strains of FMDV. Vaccine strains held in banks are generally those which are felt to have the greatest risk of introduction, based on the worldwide epidemiological situation [1]. These stocks are under continual review; important new strains are added periodically, and some stored antigens become obsolete. Strain selection is complex. The OIE/Food and Agriculture Organization (FAO) World Reference Laboratory periodically recommends and prioritizes FMDV vaccine strains for banking in FMD-free areas (in addition to separate regional recommendations for vaccines in endemic areas) [8;109]. A number of factors play a role in these recommendations, including the strains causing recent FMD outbreaks, the ability of vaccines to protect animals from other strains within that serotype, and the availability of vaccine strains within the portfolios of manufacturers that can fulfill the quality requirements for use in FMD-free regions [8]. More vaccine strains may be recommended for some serotypes than others. 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 [8;223]. Serotype A and SAT viruses are genetically and antigenically diverse, and multiple vaccine strains are needed for immunization as they must closely match the outbreak strain [8;22;223]. Serotype Asia-1 has tended to be antigenically homogeneous, and only one strain was recommended for immunization and vaccine banking as recently as 2013 [8;223]. However, new Asia-1 variants that are poorly matched with the Asia-1 Shamir vaccine strain have been recognized during recent outbreaks, and additional (or other) strains may be recommended for banking in the future [8]. One vaccine strain is also recommended for serotype C, which has not been reported since 2004 [8].

FMDV antigen concentrates can be stored in vaccine banks for many years [16;152;217]. The integrity of the antigens must be maintained while they are frozen, stored, thawed and diluted [152]. During storage, some virus particles rupture or aggregate [152]. There is little information on this phenomenon, partly because the data are proprietary and are not readily published by manufacturers; however, it is considered to be normal by manufacturers if, with highly purified antigens, 10% of the initial virus particles are lost within the first five years of storage [152]. After 14 years, as much as 40% of the antigen mass may be lost in some cases [152]. The stability of FMDV antigens seems to be strain- dependent [152]. The OIE recommends testing samples for the integrity of the antigens or acceptable potency of the final product at appropriate intervals, currently recommended to be every 5 years [16]. Some tests that may be used include 146S quantification, vaccination serology or challenge studies.

In an outbreak, stored antigens from banks can be formulated rapidly into complete vaccines. These vaccines can be tailored to the epidemiology of the outbreak [109;152]. Banks are usually able to make either monovalent or polyvalent vaccines that contain oil or aluminum hydroxide/ saponin as the adjuvant. It is possible to adjust the potency of the vaccine according to need and to the relatedness of the field and vaccine strains. The time between receipt of the order and vaccine delivery has been estimated to be 4 to 13 days, depending on the distance the antigens and/or vaccine must be shipped, the daily finishing and filling capacity of the manufacturer, and the availability of flights [152]. At an international conference for representatives of vaccine banks, manufacturers' estimates for vaccine formulation were 3-7 days, with the period between ordering and application in the field likely to be at least 6-10 days [223].

Noncommercial vaccine banks usually operate on a relatively small scale, and a bank may be able only to meet the initial needs during an outbreak [109]. The number of vaccine doses available should be expressed in relation to the expected potency; it will vary with the amount of antigen per dose in the final vaccine. Because some stocks are duplicated in different banks, it might be possible to obtain additional vaccine supplies from other countries [1;109]. Cooperative agreements or formal reciprocal supply

agreements with other banks would facilitate such planning. However, vaccine banks must also consider whether to hold antigens in reserve for their own member countries if an outbreak were to spread [109]. In 2006, representatives of FMD vaccine banks approved the creation of an international FMD vaccine bank network, to operate under the auspices of the OIE [221;223]. Some of the goals of the network include addressing sudden increases in the demand for vaccine and establishing a global vaccine reserve for FMD, as well as harmonizing vaccine and test standardization and certification.

6.3.4 Vaccine Formulation from the North American FMD Vaccine Bank

The North American FMD Vaccine Bank contains a limited number of vaccine antigen concentrates (VACs), which are ready to be formulated into vaccines. The VACs are kept on the vapor phase of liquid nitrogen at Plum Island, New York. NAFMDVB would be activated by the joint decision of the Chief Veterinary Officers of the U.S., Canada and Mexico, and could be used for an outbreak anywhere in North America. Because the manufacture of conventional or emergency inactivated FMD vaccines is prohibited in the U.S., frozen antigens from NAFMDVB must be shipped to the country that produced the antigen, and formulated and finished by the antigen manufacturer [10]. Production from a field strain or an established master seed would take longer to formulate into vaccines than VACs. Normal batch or serial tests to demonstrate purity, safety, and potency would take several weeks to complete, if they are done before the vaccine is used [10].

6.3.5 Conventional Inactivated FMD Vaccines from Commercial Manufacturers

Countries may choose to use a commercially available conventional FMD vaccine in an outbreak, as the Netherlands did in 2001, rather than order an emergency vaccine from a vaccine bank [109]. Commercial manufacturers have larger operations, and regularly produce vaccines for countries where FMD is still endemic [1]. They can also adapt field strains to produce new vaccines if necessary. A disadvantage to relying on a commercial vaccine manufacturer is that it might already be contracted to produce vaccines for other customers. Availability was not a constraint in 2001 for the Netherlands, which was able to vaccinate immediately with a conventional vaccine after the E.U. approved the use of vaccination [1]. However, the South American quadrivalent inactivated FMD vaccine permitted for distribution in the U.S. during an outbreak is, at present, produced in sufficient quantity only to meet the needs of Biogenesis Bago's current customers [216]. The manufacturer does not maintain stocks of this vaccine that could be immediately available in sufficient quantity for rapid use in controlling even a small outbreak. It 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 in the U.S. 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.

6.3.6 New Inactivated Vaccines from Field Viruses

New vaccine strains may need to be produced for an outbreak, either because no reasonably well-matched vaccine strain is available, or to optimize vaccine efficacy. Master seed viruses (MSVs) for FMD have traditionally been made by adapting field viruses to culture, via passage in a suitable cell line [10;10;16;22;151]. The number of passages necessary to produce a high yielding, efficacious MSV differs between strains [22]. While many new vaccines have been produced successfully, some field strains do not grow well in culture, and the quality or number of field strains from an outbreak might be inadequate [10;223]. In addition, the adaptation process is time-consuming, and has the potential to result in antigenic changes during adaptation and *in vitro* growth [10;22;224;225].

An experimental approach, which might mitigate some of these difficulties, involves the development of new vaccine strains by modifying cDNA clones of existing strains [225-229]. In one recent study, a vaccine was developed for a serotype A virus that does not grow well in culture, by substituting the FMDV capsid coding region into the cDNA clone of a serotype O vaccine strain [228]. In a similar experiment, partial replacements of genetic material were made between field and vaccine strains of SAT

viruses [229]. 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 [225].

If the adaptation of a field strain to culture is successful, the lead time for vaccine preparation is 1 to 6 months, depending on how readily the strain grows *in vitro*, its yield and immunogenicity, and the tests that must be conducted [1;223]. If the new vaccine is needed quickly, an emergency release may allow it to be used without necessarily finishing all aspects of control testing.

6.3.7 Experimental Vaccines: Inactivated Vaccines with Marker Deletions, and Safer Platforms for Inactivated Vaccine Production

6.3.7.1 Inactivated Vaccines with Marker Deletions

Inactivated FMD vaccines with marker deletions in nonstructural proteins (i.e., 3A) [230] or capsid proteins [231;232] have been investigated as a means to identify animals that become infected after vaccination. Such vaccines would be used with companion diagnostic tests targeted to the protein that was altered in the vaccine. Some of these vaccines have been evaluated experimentally in pigs [230] or cattle [231;232] and appear promising. However, more extensive evaluation will be necessary to compare their efficacy to unmodified vaccine strains [154;232].

6.3.7.2 Leaderless, Inactivated FMDV Vaccine Constructs

An FMDV construct termed FMD-LL3B3D, which has a deletion in the leader protease (Lpro) gene and two marker mutations in the 3B and 3D NSPs, is in development in the U.S, as a safer platform for the production of inactivated vaccines [233]. Ideally, the leader deletion results in viruses that can still replicate in culture, but do not cause disease in FMD-susceptible animals. Inactivated vaccines produced from these constructs would share many of the characteristics of standard inactivated FMD vaccines, but with increased safety during manufacture. 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. The marker mutations in the 3B and 3D proteins would allow serological reactions to NSPs in field viruses to be distinguished from reactions to the vaccine strain, even when the vaccine is unpurified [233]. The FMD-LL3B3D backbone contains unique restriction endonuclease sites on either side of the capsid coding region, which would allow these structural gene sequences to be changed readily [233;234].

The initial construct (FMD-LL3B3D A_{24} Cruzeiro) codes for a serotype A_{24} Cruzeiro virus [233;234]. In cattle, an oil adjuvanted vaccine produced with this construct prevented clinical signs and detectable viremia, when the animals were challenged 3 weeks after receiving a single dose [233]. Neutralizing antibodies were found in some animals by day 7, suggesting that the vaccine might also be protective sooner. A proprietary adjuvant is also being investigated, and constructs that utilize the same backbone are being developed for other FMDV strains and serotypes.

6.3.8 Immunity after Infection Compared to Vaccination with Inactivated Vaccines

Humoral immune responses, with the production of neutralizing antibodies, are generally correlated with recovery from infection with FMDV and resistance to reinfection [19;22;235-237]. Cell-mediated immune responses (CMI) have also been reported in FMDV infected animals, although the role of this form of immunity is still under investigation [19;236;238]. Mucosal immune responses, with the production of IgA, might also play a role in protection [19;235].

Inactivated FMD vaccines are thought to protect animals by inducing humoral immunity, although there is some evidence that they may also stimulate some degree of CMI [19;21;239;240] possibly as the result of cross-priming [241]. Inactivated FMD vaccines are not thought to result in any mucosal immunity

[19;235;242], with the possible exception of certain highly potent vaccines, given repeatedly ([243;244] cited in [19;22]).

6.4 Vaccines Manufactured without Live Virus

The restrictions on live FMDV in the U.S. increase the desirability of other types of vaccines, which could be manufactured domestically. FMD vaccines that can be made without live virus can be produced and licensed in the U.S. [10]. Some of these vaccines may also be able to stimulate improved CMI responses to FMDV, compared to inactivated vaccines.

6.4.1 Conditionally Licensed Replication-defective hAd5-vectored FMD Vaccine

Vectors generated from human adenovirus 5 (hAd5), a mild respiratory pathogen of people [245], have been tested in a number of experimental vaccines and gene therapy constructs for animals and humans. The replication-defective hAd5 construct used in FMD vaccines is a live vector that lacks three regions of the adenovirus genome necessary for virus replication [10]. As a result, it cannot produce new adenoviruses except *in vitro,* within cell lines that have been engineered to contain certain complementation functions [246]. When a vaccine construct is transfected into such a packaging cell line, 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 animal species and become internalized [247-249] cited in [246]; however, they cannot replicate and infect additional cells. Once the virus particle enters the cell, the vaccine construct is transported to the nucleus and transcribed. The hAd5-vectored FMD vaccine construct encodes all of the FMDV capsid proteins, as well as a few NSPs (2A, 3C and sometimes 2B) necessary to generate these proteins from the viral precursor polyprotein [10;155;156;246;250-253]. The result is the expression of FMDV capsid proteins in the animal, and their assembly into "empty capsids," which do not contain infectious nucleic acids. The hAd5 vector does not integrate into the host genome, and the expression of vaccine proteins is transient.

Most research has been performed with a construct that encodes the serotype A_{24} Cruzeiro capsid proteins [10;156;224;246;250;253-256]. 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 the U.S.[197]. Some completed steps include production and characterization of a master seed virus, master cell line production and characterization, the establishment of a scalable manufacturing process for vaccine production, technology transfer to a CVB-licensed manufacturing facility and the receipt of regulatory approval for an outline of production [253].

The company is also developing hAd5-vectored FMD vaccines for other serotypes and strains, to follow conditional and full USDA licensing programs [253]. New vaccines can be generated in this system by replacing the capsid coding sequence in the hAd5 vaccine construct. Theoretically, this could produce effective vaccines for a variety of FMDV serotypes and strains, including field strains that have not been adapted to cell culture [10;224;253]. In practice, some of these constructs might be less effective than the A_{24} Cruzeiro vaccine, at least using the original vector. Early experiments with serotype O vaccines (which require higher antigen doses in conventional vaccines [10;19;137;219]) did not demonstrate sufficient protection in pigs [251;257]. 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 [257]. 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} Cruzeiro vaccine in previous experiments.[251] 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 [253]). Full evaluation of each hAd5 construct for U.S regulatory approval would be expected to take 3-5 years [10] unless a conditional license is issued. An entire program for the licensure of 10 separate single master seeds expressing relevant FMD constructs is under consideration as a 5-6 year program.

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 inactivated vaccines, the degree of protection would probably be greater within some serotypes (e.g., serotype O) than others (e.g., serotype A).

6.4.1.1 Production and Storage of Adenovirus-vectored FMD Vaccines

Because no live FMDV is involved, hAd5-vectored FMD vaccines can be manufactured in the U.S, and high biological containment facilities are not needed [224]. Experimental batches of these vaccines are already being made on the U.S. mainland under BL2 conditions.

Human Ad5-vectored FMD vaccines are made as ready-to-use products [10]. Preliminary studies suggest that hAd5-vectored FMD vaccines will be very stable for years in the frozen state [253]. One estimate of the shelf life, when stored frozen, is at least 3 years [10]. 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 [253].

6.4.1.2 Use of hAd5-vectored Vaccines with NSP DIVA Tests

The current hAd5-vectored FMD vaccine platform contains only the capsid proteins and the nonstructural proteins 2A, 3C, and in some cases 2B [10;156;224;246;250;253-256]. These vaccines can be used with a variety of DIVA tests including the 3ABC ELISA ([10;155;258]. Although the 3C protein is produced by the vaccine, seroconversion does not seem to occur in this assay [258]. Rare false positives have been identified among animals; however, these animals have always been seropositive before vaccination [258]. DIVA tests would need to be validated for use with this vaccine in surveillance.

6.4.1.3 Potential Interference by Antibodies to the Vector

Immune responses to the adenovirus vector might limit the vaccine's efficacy if there is pre-existing immunity to other hAd5-vectored vaccines, or if multiple doses must be given [259]. Several studies have detected antibodies to this vector in cattle and pigs immunized with hAd5-vectored FMD vaccines [250;253;255;260]. An experiment in pigs indicated that pre-existing immunity might be a concern, when the vaccine was given 2 weeks after injecting the vector alone [250]. In cattle, titers to the vector tend to peak 2 weeks after vaccination, and a second dose ofhAd5-vectored FMD vaccine, given after the titers had declined, was able to boost the immune response to FMDV [10].

6.4.1.4 Immune Responses Induced by hAd5-vectored Vaccines

Live vectored vaccines 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 CMI (measured as cytotoxic T cell responses) [238]. There is no published evidence that these vaccines induce mucosal immunity after parenteral inoculation.

6.4.2 Experimental Vaccines Manufactured without Live Virus

6.4.2.1 Alphavirus-vectored FMD Vaccines

FMD vaccines based on an alphavirus "replicon" vector (from the TC-83 vaccine strain of the Venezuelan equine encephalitis virus) are in development. 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 [261;262]. These constructs replicate in the cytoplasm of infected cells in the animal, and can provide high levels of antigen expression [261-265]. Alphavirus replicon vectors are usually delivered to the cell by packaging the vector construct into virus-like particles, and they cannot produce new virions and spread to other cells

[261;263;264;266]. The FMD vaccines in development in the U.S. are intended for the production of a variety of viral strains and serotypes. To date, two vaccines have been constructed [267]. The first vaccine encodes the capsid and 3C coding regions of the A_{24} Cruzeiro strain of FMDV [267]. Information presented at a recent conference, and additional unpublished research, suggests that this vaccine can induce humoral immunity and protects cattle from challenge [267]. A second vaccine that contains serotype SAT-2 has also been tested, but no information has been published as of May, 2015.

6.4.2.2 Plasmid DNA Vaccines

DNA vaccines consist of plasmids that encode the genes for vaccine proteins, together with the elements needed for gene transcription and sequences required for plasmid replication during the manufacturing process in bacterial cell cultures [268]. When DNA vaccines are injected into an animal, some of the plasmids are taken up by cells and reach the nucleus, where the genes they carry are transcribed and translated [269]. Only a small proportion of injected DNA is ordinarily taken up by cells [261;270;271]; thus, plasmids are often administered by techniques such as electroporation or particle bombardment ("gene gun") to improve uptake and vaccine potency [240;261;268;272-274]. By incorporating only selected viral genetic sequences for specific proteins (e.g., FMDV capsid proteins), DNA vaccines could be used with DIVA tests. Some reports in the literature have described complete clinical protection of pigs, cattle or sheep immunized with DNA vaccines and challenged with FMDV, using various approaches [268]. A DNA vaccine for FMD is currently being developed in the U.S., but there is little information about this vaccine. In a conference presentation, pigs were reported to develop antibodies to FMDV proteins after vaccine administration by electroporation [275].

6.4.2.3 Other Experimental Vaccines and Approaches

Several other viral vectors (e.g., pseudorabies virus, poxviruses) have been investigated as methods to deliver FMD capsid genes to the animal, although none of these candidates is currently as well-characterized as the hAd5 system [224].

Subunit vaccines based on FMDV proteins and peptides have also been investigated. [155;224]. Capsid-based peptide vaccines were sometimes protective in rodent models; however, they have not been consistently protective in cattle and pigs in published reports [10;224]. Some new developments (e.g., dendrimeric peptides) appear to be promising in improving immunogenicity and protection from challenge [224]. Peptide vaccines induce narrow immune responses, and viral variants can evade the immune response if a limited subset of epitopes is used [163;224]. For this reason, these vaccines might provide selection pressure for the evolution of FMDV variants. Subunit and peptide vaccines could be used with serological DIVA tests, and no live virus would be required during manufacturing.

Virus-like particles (VLPs) are formed by the self-assembly of capsid proteins. Various expression systems have been used to produce VLPs, including mammalian cell lines transiently or stably transfected with the viral expression vector, baculovirus/insect cell or baculovirus/larva systems, yeasts or bacteria, and plant-based expression systems [224]. Most VLP-based FMD vaccines have not yet been tested for efficacy in cattle or swine [224]; however, one baculovirus-derived vaccine was protective against homologous challenge in cattle [276].. VLP vaccines would not require the use of live virus during manufacturing, and could be used with serological DIVA tests.

7. VACCINE MATCHING, POTENCY AND SAFETY

Summary

Genetic characterization can suggest that a new strain has emerged and needs to be matched with a vaccine, or that the field virus is genetically close to one that already has vaccine matching information. It may fail to accurately predict the presence or absence of *in vivo* cross-protection between some viruses.

Vaccine matching is used to determine whether a given vaccine is likely to provide good protection

against a field strain. Vaccine matching and potency testing are used in concert, as more potent vaccines are more likely to be more effective against less closely related strains. The selection of potential vaccine strains to match should be based on the serotype of the field virus, its region of origin and any other information on its characteristics. Vaccination and challenge studies in the target species can determine both the potency of the vaccine and its cross-reactivity with the field strain, and are the most reliable method of matching. Such studies are frequently impractical, because a decision for emergency vaccination must often be made quickly. *In vitro* serological tests can also be used for vaccine matching, and generate results rapidly. The OIE recommends that the two dimensional virus neutralization test be used. Matching by ELISA has also been described; however, the OIE currently recommends its use only for screening. The 'r' value indicates the closeness of the match in serological tests, with $r1 > 0.3$ in the VNT suggesting that a potent vaccine is likely to be protective. Matching by serological tests cannot account for differences in vaccine potency. If r1 suggests that a vaccine strain does not provide a sufficient match for the field virus, a heterologous cross-protection challenge test can be conducted. The version of this test described in the OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals requires at least 2 months to conduct. Alternatives are to match the field isolate against other vaccine strains, or adapt a field virus to produce a new vaccine.

Higher potency vaccines result in a faster onset of immunity and less virus shedding. They are also thought to provide better protection against heterologous strains of FMDV within the same serotype, although this might vary with the strain. Boosters are an alternative to increase vaccine efficacy, and can also improve the breadth of antigenic cover by increasing the amount of cross-reactive antibodies. However, immunity develops more slowly than if a single dose of a highly potent vaccine is used, and protection against heterologous strains is not expected to last as long as with a well-matched vaccine. If the antigenic differences between the vaccine and field strain are large, only a new vaccine strain is expected to provide reasonable efficacy. Formulating vaccines with higher potency may result in fewer doses if the antigen amount is limited, and it may be more expensive.

Potency tests include dose response studies in animals (the PD_{50} value and PGP tests), indirect tests such as serological assays (e.g., VNT or ELISA), and the expected percentage of protection (EPP) test. Each test has advantages and disadvantages. Due to the inherent variability in tests, vaccines with the same measured potencies may provide different levels of protection. Higher antigen levels usually indicate that the vaccine is more potent, but the amount of antigen needed to reach a specific level of potency varies with the strain. In some cases, increasing the antigen dose might not provide additional benefits.

Potency tests in cattle can be considered adequate evidence of vaccine quality for other species; however, consideration should be given to testing vaccines directly in a target species when the vaccine is primarily intended for use in that species.

Safety assessments for vaccines vary with the type of vaccine (inactivated or live, bacterial or viral), the adjuvants used, and the history of similar products in use, as well as the dose, vaccine claims, usage regimen and animal factors such as the species. Safety concerns include both manufacturing errors and user errors that could cause problems. Good manufacturing practices and quality control are critical. Completely inactivated vaccines and subunit vaccines are generally considered to be low-risk for animal safety. Live genetically modified organisms or vectored vaccines usually have higher-risk profiles; however, no risks have been identified in initial safety studies for the licensing of hAd5-vectored FMD vaccines. Adjuvants and other vaccine ingredients may cause local or systemic reactions in some animals. Hypersensitivity reactions have been documented with inactivated FMD vaccines, but are uncommon when the vaccine contains purified components and is inactivated with binary ethyleneimine.

There is no evidence that the antigens in inactivated FMD viruses are a safety hazard for humans. However, local reactions from oil adjuvants or other ingredients should be addressed in label warnings.

Theoretical arguments and experiences with other hAd5 constructs in people suggest that hAd5-vectored FMD vaccines used in livestock will not be a human safety concern.

7.1 Vaccine Matching

Vaccine matching is the procedure used to quantify the antigenic relationships between FMDV strains. It is used to determine whether a given vaccine is likely to provide good protection against a field strain. Vaccine matching and potency testing (described below) are used in concert, as more potent vaccines are more likely to be effective against less closely related strains.

The most reliable method of matching is to conduct vaccination and challenge studies in the target species [16]. Challenge studies can determine both the potency of the vaccine and its cross-reactivity with the field strain [16]. However, these studies require the use of live virus, animal testing and facilities for Containment Group 4 pathogens. They are also slow and expensive; it takes at least 1 month to test existing vaccines against the field strain by this method [277;278], and at least 2 months by the heterologous challenge method described in the most recent (2014) OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals [16]. During an outbreak, this may be impractical.

In vitro alternatives can be used at various stages during the matching process. Genetic characterization using sequence analysis of the P1 region (the capsid precursor polypeptide) of the FMD genome, and antigenic profiling of the field virus can suggest that a new strain has emerged and needs to be matched with a vaccine, or that the field virus is genetically close to one that already has vaccine matching information [10;16]. However, some serotype O or A strains without major antigenic differences predicted by P1 sequencing have not been cross-protective during *in vivo* heterologous challenge [279;280]. Conversely, serotype A strains can have multiple amino acid changes that do not affect antigenicity [280]. Other genetic/ antigenic approaches that have been investigated include antigenic cartography [280], or combining P1 sequence information with additional structural information on amino acid locations [281].

In vivo protection from FMDV is generally correlated with antibody titers, and serological tests can be used for vaccine matching [219;282-284]. Virus neutralization (VNT) may be the most relevant test for protection in the animal, and it is currently the serological method of choice, according to the OIE [16]. The two-dimensional (checkerboard) titration method is recommended for more accurate results. Matching by ELISA has also been described; however, the OIE currently recommends its use only for screening. Nevertheless, a few laboratories may still use ELISAs, either as the primary method for vaccine matching or as backup for VNT [8] (some have reported that they have better consistency and discriminatory capacity with ELISAs [285]). One advantage to *in vitro* serological tests is that they can generate results rapidly and do not require animal testing. ELISA tests also do not require the use of live virus. However, variation between batches of antisera can cause inconsistent results during serological matching, and there can also be discrepancies between ELISA and VNT results [286]. In addition, the results of serological matching do not always agree with heterologous challenge studies ([287] cited in [285]; and [279]). One reason for this is that serological tests alone cannot account for differences in the potency of each vaccine; more potent vaccines may protect animals from less closely related strains.

If serological matching is used, the field viruses must have been serotyped and adapted to grow in cell culture [16]. Multiple isolates should be tested, if possible, to account for any variability in the virus population during an outbreak [16;288]. The selection of potential vaccine strains should be based on the serotype of the field virus, its region of origin, any vaccine strains used in the region, and any other information on the virus's characteristics. The availability of sera for matching to particular vaccine strains may limit testing.

The serological relationship between the field isolate and the vaccine virus is the 'r' value. The OIE recommends one way testing (r1) using antiserum to the vaccine [16]. Two-way testing (r2) would also match using an antiserum against the field isolate. For vaccine matching by VNT (the two-dimensional neutralization test), r1 > 0.3 suggests that a potent vaccine strain is likely to be protective, while values less than 0.3 indicate that protection is less likely [16] or unlikely [8]. For vaccine matching by ELISA, r1 $\geq$ 0.4 suggests that a potent vaccine is likely to be protective, and r1 $\leq$ 0.4 suggests the vaccine is unlikely to be protective [8]. Confidence in the relatedness is related to the number of times the test is done [16]. The OIE currently suggests a minimum of 3 repetitions. (For further details, refer to the current OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals.) Comparative studies have found that r1 values can sometimes differ significantly between laboratories ([287] cited in [285]; and [288;289]).

If r1 is less than 0.3 in the VNT, the options suggested by the OIE include examining the field isolate against other vaccine strains, testing it against existing vaccines in a heterologous cross protection challenge test, or adapting a field virus to produce a new vaccine [16]. In the heterologous cross-protection challenge test, at least 7 FMD-naive cattle are vaccinated with the test vaccine and boosted 28-30 days later [16]. Another group of 7 or more cattle is also vaccinated with the same dose at this time. Both groups are challenged 30 days later with 10,000 BID_{50} (50% bovine infective dose) of the field strain to be tested. If the protection level is < 75% in the cattle vaccinated once, and < 100% in the cattle vaccinated twice, a different vaccine is recommended [16].

Currently, the OIE does not recommend the use of the Expected Percentage of Protection (EPP) method (see section 7.2, Vaccine Potency) under heterologous conditions [16]. It notes that correlations between protection and the post-vaccination titer tables generated by this method cannot be extrapolated to strains other than the homologous challenge strain [16]. Researchers in South America also suggest that the EPP must be used with caution in vaccine matching, as the serological correlation with protection against the homologous strain may not be strictly valid if the strain is heterologous, the vaccine differs in potency, or the values are evaluated at times other than 30 days after vaccination or revaccination [278]. However, they also note that indirect tests for matching, including EPP, have been correlated with *in vivo* heterologous challenge in some studies [278;290;291].

Novel tests for matching are being investigated, although they have not been thoroughly evaluated at this time. A study that examined sera from cattle vaccinated with A_{24} Cruzeiro and challenged with A/Argentina/2001 found that a high (> 10) IgG1/IgG2 ratio could predict heterologous protection in cattle with low neutralizing antibody titers by VNT, although it was not correlated with protection in animals that had high titers [292]. Another study examined sera from cattle vaccinated with A_{24} Cruzeiro and challenged with A/Argentina/2001, and found that the IgG1/IgG2 ratio was the most accurate test in predicting cross-protection, followed by heterologous IgG1, then VNT titers [293]. The r1 determination (with a cutoff > 0.3) was not more accurate than other assays in this study, and it was a poor predictor of cross-protection when samples with no neutralizing titers to the heterologous virus (which gave distorted values) were included. Although all of the tests evaluated had low sensitivity, using a combination of tests for screening and confirmation (e.g., homologous VNT titers confirmed by IgG1/IgG2 ratio) was more accurate than using an estimate of r1 alone.

7.2 Vaccine Potency

Higher potency vaccines result in a faster onset of immunity and less virus shedding [16;109;284]. Boosters 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 [16;284].

Higher antigen levels usually indicate that the vaccine is more potent [109]. However, the amount of antigen needed to reach a specific level of potency varies with the strain [223]. One study suggests that there may be a sigmoidal dose response, and above a certain threshold, increases in antigen concentration

might provide little improvement in serum-neutralizing antibody titers [294]. A recent study supports this hypothesis. When cattle were vaccinated with a very high potency FMD vaccine, a further five-fold increase in the antigen concentration provided no additional benefit [295]. In sheep and goats, one study reported that an O_1 Manisa vaccine was clinically protective and reduced or eliminated virus shedding when the antigen payload was 0.94μg; to 5μg; however, viremia was absent by both virus isolation and PCR only when the vaccine had a payload of 3.75 or 5μg [146]. Vaccines with increased antigen levels may be more expensive, and fewer doses are available from a given amount of antigen. This might be a factor in formulating an emergency vaccine from limited antigen supplies in vaccine banks. Some emergency (higher potency) vaccines may be less stable than conventional FMD vaccines, possibly due to proteases from the culture harvest that contaminate the vaccine and/or the type of formulation employed [109]. This effect has been reported for some vaccines but not others.

Potency is traditionally expressed as the number of 50 percent cattle protective doses (PD_{50}) within each dose of vaccine recommended on the label. The PD_{50} determination is a dose response study. At least three groups of cattle, with a minimum of five vaccinated animals per group, are used [16]. The groups are vaccinated with a full dose of vaccine or two different partial doses (e.g., ¼ and 1/10 dose). Two additional animals are nonvaccinated controls. All animals are challenged with 10,000 BID_{50} of the same type or subtype of virus as the vaccine strain, via intradermolingual inoculation. Challenge is performed 21 days after vaccination if the vaccine contains an aqueous adjuvant, or up to 4 weeks after vaccination if the adjuvant is oil. Unprotected animals are defined as those with lesions at sites other than the tongue. If an animal develops lesions only at the inoculation site, it is considered to be protected; however, highly potent vaccines may also prevent these lesions from forming. Because PD_{50} tests must be done under high security and use small numbers of cattle, the test is highly variable and the confidence limits are wide [109;284]. It is impossible to distinguish vaccines with a PD_{50} of 3, 6 or 10 based on the outcome of a single potency trial [201;296]. One study found that FMD vaccines with the same PD_{50} did not necessarily share a common level of protection [297]. This study also reported that the relationship between the PD_{50} per dose and percentage of animals protected was influenced by the FMDV serotype and the type of adjuvant in the vaccine. Whether this will be a practical concern with high potency vaccines is unknown. A recent meta-analysis reported no difference in clinical protection between different serotypes of vaccines in experimental studies in pigs, cattle or sheep [298].

An alternative potency test, which is used in South America, is also accepted by the OIE [16]. In the PGP (or PPG) test (percentage of protection against generalized foot infection), 16 cattle are immunized with a full dose of vaccine. These animals and a control group of two nonvaccinated cattle are challenged by intradermolingual inoculation of 10,000 BID_{50}, a minimum of 4 weeks later. Unprotected animals develop lesions at sites other than the tongue. In the PGP test, the vaccine should protect at least 75% of the vaccinated cattle. The PGP test is a more certain way to estimate the protective value of a cattle dose of the vaccine, compared to the PD_{50} test, but it does not estimate the number of protective doses in the vaccine. Goris et al. reported that the PGP test was more reproducible and repeatable than the PD_{50} test [299]. This study also indicated that more potent vaccines produce more consistent results in the PGP test [299]. To increase the statistical power of the PGP test, it has been suggested that additional animals could be included [299]. One option would be to combine the results from the initial potency test and later tests, if licensing regulations require that the vaccine be retested [299].

Potency tests in other species such as pigs, sheep, goats or buffalo are different from the cattle test or not yet standardized; however, if a vaccine passes potency testing in cattle, this is usually considered to be adequate evidence of vaccine quality for other animals [16]. Because African buffalo, Asian water buffalo, sheep and goats often have subclinical infections, the potency test in cattle may be a more reliable test of vaccine quality for these animals than a species-specific potency test based on clinical signs [16] At the same time, the OIE suggests that potency tests in the target species be considered when a vaccine is not intended primarily for use in cattle [16]. One study raised the possibility that some vaccines

might be less effective in water buffalo than cattle: a serotype O vaccine was clinically protective in 6 cattle, but only 4 of 6 water buffalo, after direct contact challenge from water buffalo [82].

Indirect potency tests such as the measurement of FMDV-specific titers by ELISA or virus neutralization have also been described. The OIE states that such tests could be accepted only if a strong correlation with protection has been demonstrated for the vaccine strain being tested, and the method has been "scientifically demonstrated" and published in a peer-reviewed journal [16]. However, serological tests or alternative tests can be used to measure potency for vaccine batch release if there is a satisfactory correlation between the test results and the *in vivo* potency test in the target species. Advantages of using serological testing include a decreased risk that live virus will escape, and benefits to the welfare of the experimental animals [297]. The test results are also more precise because serological tests can be quantified using a continuous scale, unlike challenge experiments [297]. Validation of the antigen load as a potency test has been difficult to achieve [201]. A disadvantage of indirect tests, compared to *in vivo* potency testing in cattle, is that cellular immunity is not measured [201].

The expected percentage of protection (EPP) test is a serological test that can be employed for potency testing [290]. This test estimates the probability that cattle will be protected against 10,000 infective doses after a single or boosted (single boost) vaccination [284]. The EPP test is evaluated using correlation tables, derived from vaccine challenge experiments, which associate the post-vaccination titer with the protection induced by a specific vaccine. If the EPP is <75% (using sera from 16 re-vaccinated animals) or <70% (using sera from 30 re-vaccinated animals), this suggests the vaccine will not protect well against the field strain. To generate the EPP tables, the vaccine must be tested in hundreds of cattle, and panels of antisera must be available [284]. In Argentina, this assay (using ELISA titers) has partly replaced the PGP test for cattle ([300] cited in [290]). The PGP challenge test is still used during vaccine licensing, or when new strains are included in a vaccine. One study reported that the EPP test was less variable than the PGP assay for the same A_{24} Cruzeiro vaccine [290]. A high degree of concordance was reported between mean EPP values from virus neutralization or ELISA testing and the PGP test [290]. The EPP based on VNT was more variable than EPP based on ELISA, and falsely rejected the vaccine batch on one of 10 occasions [290].

7.3 Potency and Other Factors Affecting Cross-Protection between Strains

Higher potency vaccines are thought to provide better protection against heterologous strains of FMDV [109;210;223;279;284], by increasing the titers of cross-reactive antibodies [19]. Initial studies reported at a meeting for representatives of vaccine banks suggest that this effect occurs with some but not all strains [223]. In a recent study, a 10-fold higher antigen dose in a serotype A vaccine, administered to cattle, resulted in a 4-fold increase in titers against all 10 heterologous serotype A strains tested [210]. Boosting a less potent vaccine can also improve the breadth of antigenic cover [16;284]. However, repeated vaccination cannot overcome large antigenic differences; in this case, only a new vaccine strain is expected to provide reasonable efficacy [22]. Immunity against heterologous strains generated by boosting is not expected to last as long as when the vaccine is well-matched [19;301].

In one recent study, only higher antigen doses resulted in better cross-reactivity; neither the choice of adjuvant (oil vs. aluminum hydroxide/saponin) nor route of administration (intradermal vs. subcutaneous) had a significant effect [210]. This study also examined a previously reported phenomenon, where including two strains in a vaccine, rather than one, resulted in an improved heterologous response against a third strain. It found that a bivalent vaccine containing two strains with narrow heterologous responses stimulated better responses to some FMDV strains, but poorer responses to others, compared to vaccines containing only one of the two strains. Overall, there was no improvement. Based on these results, using a single strain that has broad coverage appears to be a better choice for protection against heterologous FMDV strains, compared to mixing strains with narrow coverage.

7.4 Vaccine Safety

In general, safety assessments for vaccines vary with the type of vaccine (inactivated or live, bacterial or viral), the adjuvants used, and the history of similar products in use, as well as the dose, vaccine claims, usage regimen and animal factors such as the species [302]. The 'worst case' scenario is usually assessed even if it is unlikely, assuming that the product will be used at its maximum potency and quantity, in animals of the highest sensitivity. Safety concerns include both manufacturing errors and user errors that could cause problems. For example, viruses in an incompletely inactivated vaccine could harm the animal or spread to other animals [302]. This was a concern with early formaldehyde-inactivated FMD vaccines, and resulting in switching to aziridine inactivation in the 1970s [10;151]. There are no reports of failed inactivation with modern FMD vaccines when good manufacturing practices and quality control are practiced [10]. However, these measures are critical. Type C outbreaks in Kenya [303] and FMDV viruses circulating in China and eastern Russia in 2005 [201] may have been linked to vaccine strains from improperly inactivated vaccines.

Completely inactivated vaccines and subunit vaccines are generally considered to be low-risk for animal safety, although adjuvants and other vaccine ingredients may cause local or systemic reactions in some animals [302]. Granulomas, abscesses, inflammation and necrosis or fibrosis may occur at the injection site. Fever, lethargy, anorexia, arthritis, soreness and decreased milk yield are also possible. A number of reports described allergic reactions (some serious or fatal) in animals immunized with FMD vaccines during the 1970s ([304] cited in [22]). 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 [22]. 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 [22]. Hypersensitivity reactions are reported to be unlikely with vaccines that contain purified components and are inactivated with binary ethyleneimine [22], although reactions (including severe reactions) are still reported occasionally. One such event occurred in Israel in 2001, when necrotic dermatitis, decreased milk yield and other adverse reactions were seen in 10-15% of the animals in a dairy cattle herd, 8 days after the annual FMD vaccination [305]. In the Middle East, where high producing dairy herds may be intensively vaccinated with FMD vaccines (e.g., vaccination with 8 strains every 10 weeks), cattle may develop unusual, severe reactions with swelling of the tongue and shedding of most of the tongue epithelium if they are infected [71]. This reaction is also thought to be a hypersensitivity reaction.

Live genetically modified organisms or vectored vaccines are generally considered to have higher-risk profiles than inactivated vaccines [302]. Initial safety studies have been completed for the hAd5-vectored A_{24} Cruzeiro vaccine [197]. One concern with some viral-vectored vaccines is the possibility that replication-competent viruses could be generated, resulting in disease. Theoretical considerations suggest that this will not be an issue with these constructs. The FMDV viral sequences are cloned into the essential E1 region of the adenovirus genome, and if homologous recombination occurred with a wild type virus (which is considered unlikely), it would produce a replication-competent human adenovirus without FMDV genetic material [246]. Wild type human adenovirus 5 is not a health concern in livestock, and while it can cause mild clinical signs in people (see section 7.4.1), this virus is already common in human populations [245]. There was no evidence for reversion to virulence or vaccine transmission to naive cattle or pigs in contact, during the risk assessment for hAd5-vectored A_{24} Cruzeiro in vaccine licensing studies [253]. 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 [253]. Whether hAd5-vectored FMD vaccines could cause allergic reactions in repeatedly vaccinated animals is not known.

Contamination of vaccines by extraneous pathogens could also cause morbidity or mortality with either type of vaccine [302]. This hazard is controlled by quality assurance steps during vaccine production. Consideration should be also given to the possibility of interactions with other vaccines [302]. This does

not seem to be an issue for inactivated FMD vaccines, which have been administered simultaneously with many other vaccines including rabies, anthrax and porcine parvovirus, with no apparent effect on either vaccine ([306-309] cited in [22]).

7.4.1 Risks to Humans during Vaccine Administration

Risks to people who administer or contact FMD vaccines should also be assessed. There is no evidence that the antigens in inactivated FMD viruses are a safety hazard for humans [310]. However, local reactions from oil adjuvants or other ingredients should be addressed in label warnings [302].

Theoretical arguments and evidence from the use of similar vectors suggest that hAd5 vectored-FMD vaccines are also expected to have little or no risk for people. The vector in these vaccines is based on human adenovirus 5, a pathogen that causes mild, self-limiting respiratory disease or inapparent infections in immunocompetent individuals (mainly children), and can cause conjunctivitis after inoculation into the eye [245]. The vector itself is not expected to be a concern: it does not contain the structural genes for human adenovirus 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 [246]. Because exposure to adenoviruses is common among children, the presence of such a construct in the environment is not expected to be a concern [246]. Adenoviral constructs and adenoviruses have also been tested or used in humans for a number of years [245]. Live human adenovirus 5 vaccines have been tested by enteric administration, without adverse effects. Trials with various hAd5-vectored constructs, administered by parenteral routes, have also been conducted in people. Concerns about the 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, and when conditionally replicating adenoviruses are used [245;311]. Adenoviruses are very effective inducers of interferon and innate immune responses, and these responses can result in unexpected adverse effects [259]. 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 [311].

8. VACCINE WITHDRAWAL TIMES IN MILK AND MEAT

Because vaccination does not usually result in harmful residues or immune responses that differ from natural immune responses, countries do not necessarily require a withdrawal period for the antigen component in a conventional vaccine, unless it is a live virus zoonotic agent [302]. Other vaccine components such as adjuvants and excipients must also be considered in the safety evaluation, and may require withdrawal periods [302]. Prior experiences with these components in other vaccines should be considered [302]. In the U.S., withdrawal times before animals may be slaughtered after vaccination with specific products are established by the USDA Center for Veterinary Biologics, and will be found on the vaccine label. 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. The proposed withdrawal time for the hAd5-vectored FMD A_{24} Cruzeiro vaccine, which does not include an adjuvant that would cause local inflammation, is 21 days. Because of local injection site inflammation, oil-adjuvanted FMD vaccines all have a 60 day slaughter withholding time. There are no post-vaccination milk withholding requirements for FMD vaccines, but vaccination does tend to cause a transient decrease in milk production.

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 [310].

9. VACCINES AND DIVA TESTS AVAILABLE IN THE U.S.

In 2007, the National Veterinary Stockpile FMD Countermeasures Working Group (FMDCWG) conducted an in-depth analysis of available measures to control and eradicate FMD if an outbreak were to

occur in the U.S. [10]. They recommended that the North American FMD Vaccine Bank stockpile monovalent and multivalent, finished, highly purified and DIVA compatible FMD vaccines with well-characterized ingredients, high potency, and fully demonstrated purity, safety, potency and efficacy. FMDCWG recommended that oil emulsion vaccines be stocked. Vaccine antigen concentrates for two new vaccine strains should be added to NAFMDVB every year. They also recommended the development and licensing of hAd5 vectored-FMD vaccines. FMDCWG recommended that the U.S. stockpile (e.g., establish contracts to deliver) commercial 3ABC test kits (Ceditest [now PrioCHECK] ELISA) and a laboratory-based, high–throughput, NSP serological test for use during an outbreak where vaccination is employed, as well as tests to detect cases in an outbreak where vaccination is not used [10]. The PrioCHECK ELISA is fully validated in cattle, and confirmatory testing has been completed for its use with hAd5-vectored FMD vaccines [10]. An additional advantage to this test is that it is a blocking ELISA and can be used with any species [173].

10. EFFECTS OF VACCINATION ON VIRUS TRANSMISSION

Summary

The main purpose of emergency vaccination is to end or reduce virus transmission. This can be accomplished by increasing the minimum infectious dose of virus, and/or decreasing virus shedding from animals that become infected.

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). A limited number of transmission studies for FMD have been conducted in experimentally infected, vaccinated animals. To date, transmission studies for 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 theoretically unlikely. Movement controls and quarantines also decrease transmission between farms. For these reasons, Rh values are expected to be lower than R0 values.

Experimental studies that have examined the effects of inactivated vaccines on transmission suggest that:

- Vaccination can reduce FMDV transmission in cattle and sheep [49;98;139;140;207;312]. In some cases, immunization with a potent vaccine may decrease the estimated value of R to less than 1 [49;98;139;140]. Some vaccines may be more effective than others [207]. In ruminants, vaccination reduces virus shedding in oropharyngeal secretions and milk, as well as decreasing viremia [49;72;137-140;142;143;146;147;201;207;279;313-316]. Occasionally, vaccines can completely prevent virus shedding in some individual cattle, sheep or goats in an experiment [140;146;295;316]. A recent meta-analysis of published and unpublished experiments found that, in addition to protecting cattle and sheep against clinical signs, the risk of infection was 0.71 times lower in vaccinated than nonvaccinated cattle, and 0.59 or 0.68 times lower (depending on the analysis) in vaccinated than nonvaccinated sheep [298].
- Vaccination reduces virus shedding in pigs in some experiments [72;113;242;317-319]. Some experimental and field studies have also reported that vaccination can decrease virus transmission to contacts [242;317;319-322]. One study found that R remained above 1 and vaccination was not sufficient to prevent an outbreak if the challenge was severe, although the transmission rate was reduced [141]. A recent meta-analysis of published and unpublished experiments found that, in addition to protecting pigs against clinical signs, the risk of infection was 0.67 times lower in vaccinated than nonvaccinated swine [298].

- Some studies suggest that vaccination is more effective in reducing virus shedding and transmission when the interval between vaccination and challenge is longer [128;242;312;317;319]. (See also "onset of immunity" below.)

Experimental studies with hAd5-vectored A_{24} Cruzeiro vaccine found that this virus could reduce or eliminate transmission between cattle [253]. Transmission studies in pigs have not been published, but virus shedding is reduced [156;250].

Vaccines may perform better in experimental animals than in the field. Research animals are usually in optimal health and on a high nutritional plane, concurrent diseases are generally absent, and vaccine storage conditions and technique are well controlled. In contrast, vaccination conditions may not be optimal in the field.

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10.1 Transmission Studies Using Inactivated Vaccines

10.1.1 Transmission Studies and Virus Shedding in Cattle

In lactating dairy cows, a single vaccination with an oil adjuvanted type O vaccine (PD_{50} of approximately 9) appeared to be capable of halting virus transmission when the animals were challenged after 2 weeks [140]. In this study, there was no virological or serological evidence that vaccinated, inoculated cows transmitted FMDV to any susceptible cows in contact, and R was 0. Nonvaccinated, inoculated cows transmitted FMDV to all susceptible contacts; R in nonvaccinated cattle was estimated to be ∞ (1.3;∞), and significantly greater than 1. Nonvaccinated cows shed virus in oropharyngeal fluid, blood and milk. In contrast, no virus or nucleic acids could be detected in oropharyngeal fluids, blood or milk samples during the acute period in any of the vaccinated inoculated animals. Despite this, 3 of 10 cows inoculated directly with the virus became carriers, when tested on days 28, 29 and/or 30. In calves, a similar experiment demonstrated that vaccination significantly reduced, but did not completely eliminate, virus transmission [139]. FMDV was transmitted from inoculated animals to susceptible contacts in all six groups of nonvaccinated calves, but only one of six groups of vaccinated calves. In calves, vaccination decreased R from 2.52 (1.13; 52.1), which was significantly greater than 1, to 0.18 (0.01; 1.2), which is significantly less than 1. The reduction in R value was statistically significant. FMDV was detected in

oropharyngeal fluids of both vaccinated and nonvaccinated calves, but only some of the nonvaccinated calves developed viremia [139]. A re-analysis of the data from this experiment estimated that R was also lower in subclinically or preclinically infected, vaccinated calves (< 0.001 [0; ∞]) than subclinically or preclinically infected nonvaccinated calves (0.30 [0.03; 3.43]) [83].

Studies reported by other groups support these findings. Cattle vaccinated with a high potency vaccine (PD_{50} of 19), 3 weeks before challenge with a relatively low dose of virus, did not transmit FMDV to susceptible cattle [312]. This study reported that vaccination 4-14 days before challenge was less effective and resulted in greater transmission; however, the challenge dose was not standardized between experiments, and the vaccine used in some of the latter experiments (which were conducted at different times) was a year old. Other studies have also found that immunization with high potency FMD vaccines can reduce, though not necessarily eliminate virus shedding [137;147;279;314]. Cox et al. reported that, if only 10 days were allowed to pass between immunization and challenge, vaccination had no significant effect on the number of subclinically infected cattle or on virus shedding overall, but 10–150-fold less viral RNA was recovered in the early period after challenge [315]. Golde et al. found that vaccination reduced virus transmission in nasal secretions when the interval between vaccination and challenge was 4 days, and no virus was detected when this interval was 7 days or longer [128]. Some experiments have also reported that conventional FMD vaccines can decrease virus shedding in cattle [72;201;313].

Estimates from various FMD outbreaks where vaccination was employed support its ability to reduce R (see section 15). There might be some benefit even in partially vaccinated herds. In Bolivia, R ranged from approximately 1 to 2.7 in herds that had been incompletely vaccinated and had become infected, a value lower than the estimated R in nonvaccinated herds [174]. A disadvantage in these partially vaccinated herds was that the effectiveness of clinical inspection was reduced, both in the increased number of animals with mild clinical signs and in the false classification of animals as FMD-positive (e.g., for lameness due to other causes) in an outbreak.

10.1.2 Transmission Studies and Virus Shedding in Sheep

In a study of virus transmission among 10-week-old lambs, vaccination with an oil adjuvanted vaccine (PD_{50} of at least 6) did not eliminate the shedding of a serotype O virus when the animals were challenged after 2 weeks, but it significantly decreased both the amount of virus shed and the duration of shedding [49]. The reproduction ratio R was estimated to be 0.22 (0.01; 1.78) in vaccinated lambs, and 1.14 (0.3; 3.3) in nonvaccinated lambs. The difference between these R values did not reach statistical significance, probably because too few inoculated lambs became infected in the vaccinated group, and transmission to contact animals could not be evaluated from uninfected lambs. This experiment also suggests that the effect of vaccination on transmission in sheep might be small, since R is low even in nonvaccinated lambs, and movement controls might be sufficient to stop virus transmission in this species [49]. A re-analysis of this data found that R was low in both nonvaccinated (0.21 [0.02; 2.48]) and vaccinated (0.16 [0.009; 2.96]) lambs that were subclinically infected or incubating the disease [83]. In a subsequent transmission study under similar conditions, an Asia-1 Shamir vaccine (oil adjuvant) did not prevent vaccinated 10-week-old lambs from becoming infected with a poorly matched (r1 = 0.1) Asia-1 virus (Asia-1/Turkey 11/2000), when the animals were challenged 2 weeks later [98]. However, these lambs did not transmit the virus to any vaccinated lambs, while R was estimated to be 1.14 [0.3; 3.8] in nonvaccinated lambs. The difference between the 2 groups was not statistically significant.

In another study where the interval between vaccination and challenge was 3 to 10 days, transmission between sheep was reduced or prevented by either oil or aqueous formulated C_1 Oberbayern vaccine, but animals vaccinated with oil formulated Asia-1 India vaccine transmitted a homologous virus to susceptible contacts [207]. A study conducted in both sheep and goats reported that vaccination with an oil adjuvanted type O vaccine decreased average virus shedding in nasal secretions by 100-fold and in oropharyngeal secretions by 1000-fold compared to nonvaccinated animals [316]. In this experiment,

FMDV could not be isolated from any vaccinated animal, but viral RNA was sometimes found. Vaccination was also reported to decrease virus shedding from sheep and goats in other studies [138;142;143;146;207], and vaccines with higher antigen payloads decreased virus replication more than vaccines with smaller amounts of antigen [138;146].

10.1.3 Transmission Studies and Virus Shedding in Swine

In pigs, the effect of vaccination appears to vary significantly with the study conditions, particularly the severity of challenge, vaccine dose and time before challenge. Vaccination may be less effective in preventing virus transmission among this species than in ruminants. Eble et al. reported that, in pigs vaccinated 2 weeks before challenge, FMDV was not transmitted to susceptible contacts, virus shedding was significantly decreased, and the R value was significantly lower than in nonvaccinated pigs [242;319]. When pigs were vaccinated 7 days before challenge, the virus was transmitted to other pigs, virus shedding was seen, and most pigs developed generalized lesions similarly to nonvaccinated animals [242]. A meta-analysis of this and other experiments from this group [325] suggested that, in pigs challenged after 7 days, R was significantly reduced in pigs vaccinated with a four-fold-dose of vaccine, but not in pigs vaccinated with a single dose [320]. In another study from this group, pigs challenged 4, 5 or 6 days after vaccination excreted less virus than nonvaccinated pigs, although local virus replication was not prevented and the virus was transmitted to susceptible contacts [318]. Orsel et al reported that, with a severe challenge, vaccination did not reduce virus shedding compared to nonvaccinated animals [141]. The R value was lower in vaccinated pigs (2.42 [0.9; 6.9]) than nonvaccinated pigs (∞ [1.3; ∞]), but the difference was not statistically significant. R was also lower, without reaching statistical significance, in subclinically or preclinically infected, vaccinated pigs (1.26 [0.18; 8.96]) compared to subclinically or preclinically infected, nonvaccinated pigs (13.20 [4.08; 42.68]) [83]. However, the transmission rate (β) was significantly lower between vaccinated pigs (6.84 day^{-1}) than nonvaccinated pigs (0.66 day^{-1}), suggesting that immunization might slow virus spread [141]. In contrast, Parida et al. reported that vaccination reduced virus shedding after a severe challenge, and virus replication and excretion were correlated with the severity of the clinical signs [113]. Other studies have reported that vaccination decreases virus shedding in aerosols and/ or reduces transmission [72;317]. In one of the latter studies, vaccination was effective in preventing transmission when at least 7 days passed before challenge, but not when the interval was only 4 days [317]. One study found that immunization of pigs with a potent vaccine ($PD_{50} \geq 6$) prevented virus transmission between pens (R = 0), if the pens were separated by solid walls with some narrow gaps remaining at the edges [104]. R for within-pen transmission in this situation was estimated to be 4.4, from a previous experiment [141]. Similar solid walls reduced transmission between nonvaccinated pigs in adjacent pens from an estimated R of 23 to 1.1 (0.34-2.56), but some pigs still became infected [104]. In this experiment, FMDV was isolated from the air above the pens of nonvaccinated pigs, but not vaccinated pigs.

Recent field studies in pigs suggest that vaccination might be able to suppress virus transmission sufficiently to eradicate it in isolated swine herds. According to Poulin and Christianson, FMD can be controlled in a closed pig herd by vaccination and strict biosecurity [321]. Eradication was achieved after 1 year, and the virus did not spread to other herds. Chen at al. reported similar results in one closed pig herd infected withO/Taiwan/97 in Taiwan [322]. These studies suggest that vaccination might have a significant effect on virus transmission under field conditions.

10.2 Transmission Studies using hAd5-vectored A_{24} Cruzeiro Vaccine

One experiment suggested that the hAd5-vectored A_{24} Cruzeiro vaccine can reduce or eliminate transmission, when cattle are exposed to FMDV a week after immunization [253]. 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 separate rooms with vaccinated, FMDV-naive cattle, there was no evidence for virus transmission. In a parallel experiment, transmission to nonvaccinated cattle was reduced, but not completely eliminated: one group of cattle did not transmit the virus, but the other group infected one nonvaccinated animal. A very small amount of virus was also found in the air

samples from this room, on a single day. There are no publications reporting transmission studies in pigs; however, hAd5-vectored A_{24} Cruzeiro vaccines can reduce or eliminate virus shedding in pigs, 7 or 21 days after a single dose [156;250]

11. ONSET OF PROTECTIVE IMMUNITY

Summary

Vaccination campaigns are more likely to be successful if the interval between vaccination and exposure is long enough that animals develop adequate immunity. There is a window of susceptibility before vaccines become protective. In the Netherlands (which used a conventional inactivated FMD vaccine), several outbreaks occurred 2-9 days after vaccination was performed on a farm [109;163;326]. Whenever possible, animals should be vaccinated well before exposure is expected. This may not always be feasible in an outbreak, and vaccines that rapidly induce immunity are desirable.

The onset of protective immunity is thought to be influenced by the potency of the vaccine, as well as the severity of challenge and individual animal factors [10;16;42;143]. Most early vaccine studies and studies by the manufacturer for licensing have measured vaccine efficacy by evaluating clinical signs. However, clinical protection does not necessary correspond to reduced virus shedding, and subclinically infected animals can shed FMDV. A limited number of studies have examined the effects of vaccination on virus shedding or transmission to other animals. These studies have used vaccines with different adjuvants and potency, and varying challenge conditions, and the experiments can be difficult to compare directly.

Some general conclusions from experimental studies of inactivated vaccines (see tables 11.1 below) can be made:

- Vaccination is more effective when the time between vaccination and exposure is longer [113;128;137;147;312;315;317].
- Animals that are protected from clinical signs may still shed virus or transmit the infection [49;98;113;138;139;146;147;207;295;312;315-317]. However, some vaccines can significantly reduce virus shedding and decrease or prevent transmission [49;98;113;128;138-140;142;144;146;147;207;242;312;313;315-318;327].
- Vaccination can sometimes protect cattle from clinical signs as soon as 4-5 days [137;312;327]. One study suggests that virus shedding may be decreased at this time [128]. By 14 days, other studies also suggest that virus shedding and transmission are reduced in this species [139;140;312]. With a severe challenge, Cox et al. reported only partial protection from clinical signs and a limited decrease in virus shedding at 10 days, with improved protection at 3 weeks [147;315].
- In sheep, vaccination may decrease virus shedding and/or transmission as early as 3-7 days [143;144;207;244;316]. Other challenge studies conducted 2 or 3 weeks after vaccination in sheep and goats also report decreased shedding and/or transmission [49;98;138;142;145;146]. Protection from clinical signs 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 reported as early as 3 or 4 days after vaccination [143;144;207]. Madhanmohan et al. reported fever but no vesicles in some sheep and goats challenged at 21 days, [142;145] and complete clinical protection at 21 days in other experiments, depending on the antigen dose [146].
- It appears to be more difficult to protect pigs if they are exposed to FMDV soon after vaccination. Some studies have reported complete or partial clinical protection as soon as 3-4 days after vaccination [317;318;327]; however, with more severe challenges, pigs may not be completely protected from clinical signs even after 2 weeks. Doel et al. reported that only a few pigs were protected from clinical signs when challenged 4-16 days after vaccination, but all pigs were protected if challenged at 21-28 days [137]. Similarly, Parida et al. found that, although vaccination reduced the severity of clinical signs when pigs were challenged at 10 days, most pigs

became ill [113]. When challenged 29 days after immunization, 25% of the vaccinated pigs still developed mild clinical signs. In this study, the ability of the vaccine to reduce clinical signs was correlated with its ability to decrease virus shedding. Orsel et al. also reported that some pigs developed clinical signs when they received a severe challenge 2 weeks after vaccination [141]. In this study, vaccination was unable to significantly reduce virus shedding or prevent transmission, although it did decrease the rate of virus transmission. However, decreased virus shedding and transmission have been reported as soon as 4-7 days in few studies [242;317;318]. Eble et al. found that a high dose vaccine had some effect on virus shedding transmission when pigs were challenged at 7 days, but a lower dose was not protective until 14 days [242;325].

- Few vaccine studies have challenged animals with heterologous viruses. In one study, most cattle with antibodies to a somewhat antigenically related virus ($r < 0.3$) were protected from clinical signs when challenged at 21 days; however, only 40% of the animals produced antibodies to this virus [284]. All cattle challenged with homologous virus were protected from clinical signs in this experiment. Sheep vaccinated with O_1 Manisa were protected from clinical signs and had reduced or absent virus shedding, when challenged with a heterologous virus (O/SKR/2010, from the 2010 outbreak in the Republic of Korea) after 4 days [144].
- More information is still needed. There is little or no information about protection across a variety of ages or breeds, and little is known about the onset of protection in species other than cattle, sheep and goats.

Serological tests can also provide evidence for protection. Neutralizing antibodies have been detected within 7 days in sheep vaccinated with a variety of high potency, oil or aluminum hydroxide adjuvanted, inactivated emergency vaccines, and also in some goats immunized with an oil adjuvanted vaccine [146;328]. The titers peaked in most animals at 28 days [328]. Early induction of neutralizing antibodies in sheep and goats was dose-dependent in one study [146]. Detectable titers of neutralizing antibodies have also been reported within 7 days in pigs vaccinated with high potency, oil adjuvanted, inactivated emergency vaccines, with titers peaking at 21-28 days [328].

Information about the onset of immunity for the hAd5-vectored A_{24} Cruzeiro vaccine is limited, but some cattle have been protected from clinical signs and viremia when challenge occurred 7 days after a single dose [253;254]. Neutralizing antibodies could also be detected at this time. In one experiment, there was no transmission between vaccinated cattle exposed to the virus after 7 days, and transmission to nonvaccinated cattle was reduced [253]. In pigs, protection from clinical signs and reduced virus shedding has been detected as early as 7 days, depending on the dose of vaccine and challenge virus, and neutralizing antibody titers to FMDV developed 1-2 weeks after vaccination [250;255]. The inclusion of the coding region for the NSP 2B and an optimized promoter appear to increase potency, with neutralizing antibodies appearing as soon as 4 days after vaccination [156]. During the first week, these titers were significantly higher than in animals that received the construct without 2B, although they subsequently decreased. By 3 weeks, titers in the two groups were comparable.

11.1 Tables Summarizing Experimental Studies for Inactivated Vaccines

Table 1: Cattle

Interval between vaccination and challenge (days)	Parameter	Effect of vaccination	Vaccine potency, adjuvant, strain	Challenge type	Reference
4, 8, 12, 16 or 21	Clinical signs	Protected	PD_{50} of 41; oil or Al(OH)3; O_1 Lausanne	Aerosols from pigs for 1 hour	Doel et al.,1994
4 or 7	Clinical signs	Protected	PD_{50} of 19; Al(OH)3; O BFS 1860	Aerosols from pigs for 1 hour	Donaldson and Kitching, 1989
4 or 7	Virus transmission	Transmitted	PD_{50} of 19; Al(OH)3; O BFS 1860	Aerosols from pigs for 1 hour	Donaldson and Kitching, 1989
14	Clinical signs	Protected	PD_{50} of 19; Al(OH)3; O BFS 1860	Aerosols from pigs for 1 hour	Donaldson and Kitching, 1989
14	Virus transmission	Sometimes transmitted, some virus shedding	PD_{50} of 19; Al(OH)3; O BFS 1860	Aerosols from pigs for 1 hour	Donaldson and Kitching, 1989
21	Clinical signs	Protected	PD_{50} of 19; Al(OH)3; O BFS 1860	Aerosols from pigs for 1 hour	Donaldson and Kitching, 1989
21	Virus transmission	Did not transmit, but some virus shedding	PD_{50} of 19; Al(OH)3; O BFS 1860	Aerosols from pigs for 1 hour	Donaldson and Kitching, 1989
4	Clinical signs	Protected	oil; serotype A strain 119	Direct contact with infected cattle	Graves et al.,1968
5	Clinical signs	3 of 4 cattle protected	oil; serotype A strain 119	Direct contact with infected cattle	Graves et al., 1968
7, 10 or 14	Clinical signs	Protected	oil; serotype A strain 119	Direct contact with infected cattle	Graves et al., 1968
4	Clinical signs	Decreased clinical signs	PD_{50} of 3; oil; O_1 Manisa	Needle inoculation	Golde et al., 2005
4	Virus shedding	Decreased virus shedding, no viremia	PD_{50} of 3; oil; O_1 Manisa	Needle inoculation	Golde et al., 2005
7, 14 or 21	Clinical signs	Protected	PD_{50} of 3; oil; O_1 Manisa	Needle inoculation	Golde et al., 2005
7, 14 or 21	Virus shedding	No virus in nasal swabs, no viremia	PD_{50} of 3; oil; O_1 Manisa	Needle inoculation	Golde et al., 2005
10	Clinical signs	Most protected, a few had mild signs	PD_{50} of 18 oil; O_1 Manisa	Contact with infected cattle for 5 days, different virus of same serotype	Cox et al., 2007
10	Virus shedding	Virus shedding only reduced during earliest period after infection	PD_{50} of 18 oil; O_1 Manisa	Contact with infected cattle for 5 days, different virus of same serotype	Cox et al., 2007

Table 1: Cattle (cont'd)					
Interval between vaccination and challenge (days)	**Parameter**	**Effect of vaccination**	**Vaccine potency, adjuvant, strain**	**Challenge type**	**Reference**
10	Virus shedding	Possibly decreased virus shedding during earliest period after infection, compared to 1x dose of vaccine; no effect on carriers	10x dose of vaccine above	Contact with infected cattle for 5 days, different virus of same serotype	Cox et al., 2007
14	Clinical signs (adult dairy cattle)	Protected	PD_{50} of 9, oil; O_1 Manisa	Intranasal inoculation	Orsel, de Jong, et al., 2007[1].
14	Virus transmission	No transmission, no virus shedding	PD_{50} of 9, oil; O_1 Manisa	Intranasal inoculation	Orsel, de Jong, et al., 2007[1].

1. Orsel K, de Jong MC, Bouma A, Stegeman JA, Dekker A. The effect of vaccination on foot and mouth disease virus transmission among dairy cows. Vaccine. 2007;25(2):327-35.

Table 1: Cattle (cont'd)					
14	Clinical signs (calves)	Protected	PD_{50} of 11, oil; O_1 Manisa	Intranasal inoculation	Orsel et al., 2005
14	Virus transmission	Reduced transmission, reduced virus shedding, no viremia	PD_{50} of 11, oil; O_1 Manisa	Intranasal inoculation	Orsel et al., 2005
21	Clinical signs	Protected	$PD_{50} \geq 32$; serotype A	Injection	Brehm et al., 2008
21	Clinical signs	40% of the animals had antibodies; 87% of all animals with measurable antibodies to the challenge strain were protected	$PD_{50} \geq 32$ to homologous virus; PD50 of at least 6 to challenge virus; serotype A	Injection; different virus of same serotype; r values < 0.3.	Brehm et al., 2008
21	Clinical signs	Protected	PD_{50} of 18, oil; O_1 Manisa	Contact with infected cattle for 5 days, different virus of same serotype	Cox et al., 2005

Table 1: Cattle (cont'd)					
Interval between vaccination and challenge (days)	**Parameter**	**Effect of vaccination**	**Vaccine potency, adjuvant, strain**	**Challenge type**	**Reference**
21	Virus shedding, infection	Virus shedding reduced, 17/ 20 cattle became infected, 45% carriers by RNA, difficult to isolate virus from carriers	PD_{50} of 18, oil; O_1 Manisa	Contact with infected cattle for 5 days, different virus of same serotype	Cox et al., 2005
21	Neutralizing antibodies, number of carriers	Higher titers; fewer carriers (not statistically significant)	10x dose of vaccine above	Contact with infected cattle for 5 days, different virus of same serotype	Cox et al., 2006
28-140	Clinical signs	Fewer had clinical signs, signs were less severe	Oil; crude vaccine made for experiment; O_1 strain CANEFA*-2	Intranasal inoculation	McVicar and Sutmoller, 1976
28-140	Virus shedding	Decreased virus shedding, no viremia	Oil; crude vaccine made for experiment; O_1 strain CANEFA*-2	Intranasal inoculation	McVicar and Sutmoller, 1976
28	Clinical signs	No effect	oil, PD_{50} of 1.8 (lower than OIE standards for conventional vaccine); $1/16^{th}$, ¼ or full dose; A/TUR 14/98	Injection	Moonen et al., 2004
179 (6 months)	Clinical signs	Protected	High potency (PD_{50} unavailable); oil; A_{22} Iraq	Injection	Cox et al., 2010

Table 2: Sheep					
Interval between vaccination and challenge (days)	**Parameter**	**Effect of vaccination**	**Vaccine potency, adjuvant. strain**	**Challenge type**	**Reference**
3, 4, 6 or 10	Clinical signs	No controls or vaccinated sheep had vesicles, some controls and no vaccinated sheep had fever	PD_{50} of 41; oil or Al(OH)3; O_1 Lausanne	Aerosols from pigs, 2 hours	Cox et al., 1999
3, 4, 6 or 10	Virus shedding	Fewer shed virus in nasal secretions	PD_{50} of 41; oil or Al(OH)3; O_1 Lausanne	Aerosols from pigs, 2 hours	Cox et al., 1999
3, 4, 6 or 10	Transmission	Virus transmitted to contacts	PD_{50} of 61; oil; Asia-1 India 8/79	Aerosols from pigs, 4 hours	Cox et al., 1999
4, 5, 7 or 11	Transmission	No transmission to contacts, decreased virus shedding	$PD_{50} \geq 112$; Al(OH)3; C_1 Oberbayern	Aerosols from pigs, 4 hours	Cox et al., 1999
4	Clinical signs	Protected, except fever in some	$PD_{50} > 6$; oil; O_1 Manisa	Continuous direct contact with infected pigs; different virus of same topotype	Horsington et al., 2015
4	Virus shedding	Decreased nucleic acids in nasal secretions, virus absent from oral swabs and oropharyngeal secretions (probang), no viremia	$PD_{50} > 6$; oil; O_1 Manisa	Continuous direct contact with infected pigs; different virus of same topotype	Horsington et al., 2015
4	Number of carriers	No vaccinated sheep became carriers (vs. 53% of nonvaccinated sheep)	$PD_{50} > 6$; oil; O_1 Manisa	Continuous direct contact with infected pigs; different virus of same topotype	Horsington et al., 2015
4 or 10	Clinical signs	Protected from vesicles, some had fever (milder than controls)	PD_{50} of 18; oil; O_1 Manisa	Aerosols from pigs, 9 hours	Parida et al., 2008
4 or 10	Virus shedding	Decreased	PD_{50} of 18; oil; O_1 Manisa	Aerosols from pigs, 9 hours	Parida et al., 2008
7	Clinical signs	Almost always protected	conventional vaccine, given at 3x or 6x; vaccine stored for 10 months before use; O_1 BFS 1860	Aerosols from pigs, 2 hours	Gibson et al., 1984

Table 2: Sheep (cont'd)					
Interval between vaccination and challenge (days)	**Parameter**	**Effect of vaccination**	**Vaccine potency, adjuvant, strain**	**Challenge type**	**Reference**
7	Virus shedding	Virus shedding decreased with 6x dose	conventional vaccine, given at 3x or 6x; vaccine stored for 10 months before use; O_1 BFS 1860	Aerosols from pigs, 2 hours	Gibson et al., 1984
7, 14, 21 or 35	Clinical signs	Protected, except fever in some	oil; O_1 Manisa	Contact with sheep, 24 hours	Madhanmohan, Nagendrakumar, Srinivasan, 2010
7, 14, 21 or 35	Virus shedding	Decreased in nasal secretions by 100-fold, decreased in oropharynx by 1000-fold, no viremia	oil; O_1 Manisa	Contact with sheep, 24 hours	Madhanmohan, Nagendrakumar, Srinivasan, 2010
14	Clinical signs	Protected	$PD_{50} \geq 6$; oil; O_1 Manisa	Intranasal inoculation	Orsel, Dekker et al., 2007
14	Virus shedding	Decreased	$PD_{50} \geq 6$; oil; O_1 Manisa	Intranasal inoculation	Orsel, Dekker et al., 2007
14	Clinical signs	Protected (but minimal signs in nonvaccinated sheep)	oil; Asia-1 Shamir	Intranasal inoculation with Asia-1/Turkey 11/2000 (r_1 = 0.16)	Eble et al., 2015
14	Virus shedding	Decreased	oil; Asia-1 Shamir	Intranasal inoculation with Asia-1/Turkey 11/2000 (r_1 = 0.16)	Eble et al., 2015
14	Transmission	Prevented	oil; Asia-1 Shamir	Intranasal inoculation with Asia-1/Turkey 11/2000 (r_1 = 0.16)	Eble et al., 2015
14	Number of carriers	Unchanged	oil; Asia-1 Shamir	Intranasal inoculation with Asia-1/Turkey 11/2000 (r_1 = 0.16)	Eble et al., 2015
14	Clinical signs	Protected	full dose, 1/10 or 1/40 dose of a high potency (PD_{50}=41); Al(OH)3; O_1 Lausanne	Aerosols from pigs, 4 hours	Barnett et al., 2004

Table 2: Sheep (cont'd)					
Interval between vaccination and challenge (days)	**Parameter**	**Effect of vaccination**	**Vaccine potency, adjuvant, strain**	**Challenge type**	**Reference**
14	Virus shedding	Decreased; greatest effect with highest dose vaccine	full dose, 1/10 or 1/40 dose of a high potency (PD_{50}=41); Al(OH)3; O_1 Lausanne	Aerosols from pigs, 4 hours	Barnett et al., 2004
21	Clinical signs	Protected from vesicles, fever in some	oil, single dose, either 1x or 4x antigen; O_1 Manisa	Injection	Madhanmohan, Nagendrakumar, Narasu, Srinivasan, 2010
21	Virus shedding	Shedding decreased, no viremia	oil, single dose, either 1x or 4x antigen; O_1 Manisa	Injection	Madhanmohan, Nagendrakumar, Narasu, Srinivasan, 2010
28	Clinical signs	Protected from vesicles, fever in some	oil, one booster, either 1x or 4x antigen; O_1 Manisa	Injection	Madhanmohan, Nagendrakumar, Narasu, Srinivasan, 2010
28	Virus shedding	Shedding decreased, no viremia	oil, one booster, either 1x or 4x antigen; O_1 Manisa	Injection	Madhanmohan, Nagendrakumar, Narasu, Srinivasan, 2010
21	Clinical signs	Protected from clinical signs, except at lowest dose	oil; 0.45 to 5 µg; O_1 Manisa	Injection	Madhanmohan et al., 2012
21	Virus shedding	Decreased viremia and virus shedding in nasal secretions; dose dependent	oil; 0.45 to 5 µg; O_1 Manisa	Injection	Madhanmohan et al., 2012
21	Number of carriers	Fewer or no carriers; dose dependent	oil; 0.45 to 5 µg; O_1 Manisa	Injection	Madhanmohan et al., 2012

Table 3: Goats					
Interval between vaccination and challenge (days)	**Parameter**	**Effect of vaccination**	**Vaccine potency, adjuvant, strain**	**Challenge type**	**Reference**
7, 14, 21 or 35	Clinical signs	Protected, except fever in some	oil; O_1 Manisa	Contact with goats, 24 hours	Madhanmohan, Nagendrakumar, Srinivasan, 2010
7, 14, 21 or 35	Virus shedding	Decreased in nasal secretions by 100-fold, decreased in oropharynx by 1000-fold, no viremia	oil; O_1 Manisa	Contact with goats, 24 hours	Madhanmohan, Nagendrakumar, Srinivasan, 2010
5, 14, 21 or 28	Clinical signs	Protected; except fever in goats that received single dose	oil; O_1 Manisa; 1 or 2 doses	Injection	Madhanmohan et al, 2011
5, 14, 21 or 28	Virus shedding	Decreased virus shedding, greatest effect in goats vaccinated twice at highest dose; viremia absent	oil; O_1 Manisa; 1 or 2 doses	Injection	Madhanmohan et al, 2011
21	Clinical signs	Protected from clinical signs, except at lowest dose	oil; 0.45 to 5 µg; O_1 Manisa	Injection	Madhanmohan et al., 2012
21	Virus shedding	Decreased viremia and virus shedding in nasal secretions; dose dependent	oil; 0.45 to 5 µg; O_1 Manisa	Injection	Madhanmohan et al., 2012
21	Number of carriers	Fewer or no carriers; dose dependent	oil; 0.45 to 5 µg; O_1 Manisa	Injection	Madhanmohan et al., 2012

Table 4: Pigs					
Interval between vaccination and challenge (days)	**Parameter**	**Effect of vaccination**	**Vaccine potency, adjuvant, strain**	**Challenge type**	**Reference**
2 or 3	Clinical signs	Not protected	PD_{50} > 112, oil; C_1 Oberbayern	Aerosols from pigs, 4 hours	Salt et al., 1998
2 or 3	Virus transmission	Became viremic, transmitted virus	PD_{50} > 112, oil; C_1 Oberbayern	Aerosols from pigs, 4 hours	Salt et al., 1998
4	Clinical signs	Protected initially; later developed clinical signs from exposure to nonvaccinated contacts	PD_{50} > 112, oil; C_1 Oberbayern	Aerosols from pigs, 4 hours	Salt et al., 1998
4	Virus transmission	Virus transmitted, but decreased virus shedding	PD_{50} > 112, oil; C_1 Oberbayern	Aerosols from pigs, 4 hours	Salt et al., 1998
5	Clinical signs	One of 3 pigs protected	PD_{50} > 112, oil; C_1 Oberbayern	Aerosols from pigs, 4 hours	Salt et al., 1998
5	Virus transmission	Virus transmitted	PD_{50} > 112, oil; C_1 Oberbayern	Aerosols from pigs, 4 hours	Salt et al., 1998
7	Clinical signs	Protected	PD_{50} > 112, oil; C_1 Oberbayern	Aerosols from pigs, 4 hours	Salt et al., 1998
7	Virus transmission	Not transmitted, virus shedding decreased	PD_{50} > 112, oil; C_1 Oberbayern	Aerosols from pigs, 4 hours	Salt et al., 1998
4, 8, 12, 16 or 21	Clinical signs	Protected	PD_{50} > 112, oil; C_1 Oberbayern	Aerosols from pigs, 1 hour	Salt et al., 1998
3	Clinical signs	3 of 4 pigs protected	oil; A, strain 119	Direct contact with inoculated pigs	Graves et al., 1968
5	Clinical signs	2 of 4 pigs protected	oil; A, strain 119	Direct contact with inoculated pigs	Graves et al., 1968
7	Clinical signs	Protected	oil; A, strain 119	Direct contact with inoculated pigs	Graves et al., 1968
10	Clinical signs	3 of 4 pigs protected	oil; A, strain 119	Direct contact with inoculated pigs	Graves et al., 1968
14	Clinical signs	Protected	oil; A, strain 119	Direct contact with inoculated pigs	Graves et al., 1968
4, 5 or 6	Clinical signs	Clinical signs in some	High potency, oil; O_1 Lausanne	Direct contact with pigs, 2 hours	Barnett, Cox et al., 2002

Table 4: Pigs (cont'd)					
Interval between vaccination and challenge (days)	**Parameter**	**Effect of vaccination**	**Vaccine potency, adjuvant, strain**	**Challenge type**	**Reference**
4, 5 or 6	Virus transmission	Decreased virus shedding, virus was transmitted	High potency, oil; O_1 Lausanne	Direct contact with pigs, 2 hours	Barnett, Cox et al., 2002
4-16	Clinical signs	A few animals protected; most had clinical signs	PD_{50} of 41, oil O_1 Lausanne	Aerosols from pigs for 1 hour	Doel et al., 1994
21 or 28	Clinical signs	Completely protected	PD_{50} of 41, oil; O_1 Lausanne	Aerosols from pigs for 1 hour	Doel et al., 1994
7	Clinical signs	Most pigs not protected	oil; O Taiwan	Continuous exposure to infected pigs	Eble et al., 2004
7	Virus transmission	Transmission occurred	oil; O Taiwan	Continuous exposure to infected pigs	Eble et al., 2004
7	Virus transmission	Transmission and virus shedding decreased	oil, 4x dose of vaccine above	Continuous exposure to infected pigs	Eble et al., 2007
14	Clinical signs	No generalized lesions; localized lesions at injection site in some pig	oil; O Taiwan	Continuous exposure to infected pigs	Eble et al., 2004
14	Virus transmission	Not transmitted to contacts, virus shedding decreased	oil; O Taiwan	Continuous exposure to infected pigs	Eble et al., 2004
14	Clinical signs	Not protected	PD_{50} > 6; oil; O_1 Manisa	Exposure to infected pigs	Orsel, de Jong, et al., 2007[2].
14	Virus shedding	Vaccination did not significantly reduce shedding	PD_{50} > 6; oil; O_1 Manisa	Exposure to infected pigs	Orsel, de Jong, et al., 2007[2].
14	Clinical signs	Clinical signs in some	PD_{50} > 6; oil; O_1 Manisa	Exposure to infected, vaccinated pigs	Orsel, de Jong, et al., 2007[2].
14	Virus transmission	Transmission not prevented, transmission rate decreased	PD_{50} > 6; oil; O_1 Manisa	Exposure to infected, vaccinated pigs	Orsel, de Jong, et al., 2007[2].
14	Clinical signs	1 of 10 pigs developed clinical signs	$PD_{50} \geq 6$; oil; O_1Manisa	Exposure to infected pigs	van Roermund et al., 2010
14	Virus shedding	Decreased	$PD_{50} \geq 6$; oil; O_1Manisa	Exposure to infected pigs	van Roermund et al., 2010

Table 4: Pigs (cont'd)					
Interval between vaccination and challenge (days)	**Parameter**	**Effect of vaccination**	**Vaccine potency, adjuvant, strain**	**Challenge type**	**Reference**
14	Virus transmission	Within-pen transmission decreased; Transmission to adjacent pen (with solid wood wall) prevented	PD_{50} ≥ 6; oil; O_1Manisa	Exposure to infected pigs	van Roermund et al., 2010
10	Clinical signs	81% had clinical signs, some were severe; milder clinical signs than controls	PD_{50} of 18, oil; O_1 Manisa	Exposure to infected pigs, 9 hours, different virus of same serotype	Parida, Fleming, Oh et al., 2007

2. Orsel K, de Jong MC, Bouma A, Stegeman JA, Dekker A. Foot and mouth disease virus transmission among vaccinated pigs after exposure to virus shedding pigs. Vaccine. 2007;25(34):6381-91.

Table 4: Pigs (cont'd)					
Interval between vaccination and challenge (days)	**Parameter**	**Effect of vaccination**	**Vaccine potency, adjuvant, strain**	**Challenge type**	**Reference**
10	Virus shedding	Decreased, especially in pigs that were clinically protected	PD_{50} of 18, oil; O_1 Manisa	Exposure to infected pigs, 9 hours, different virus of same serotype	Parida, Fleming, Oh et al., 2007
29	Clinical signs	25% had mild clinical signs	PD_{50} of 18, oil; O_1 Manisa	Exposure to infected pigs, 9 hours, different virus of same serotype	Parida, Fleming, Oh et al., 2007
29	Virus shedding	Decreased shedding	PD_{50} of 18, oil; O_1 Manisa	Exposure to infected pigs, 9 hours, different virus of same serotype	Parida, Fleming, Oh et al., 2007

11.2 Tables Summarizing Experimental Studies of hAd5-vectored A_{24} Cruzeiro Vaccines

Table 5: Cattle – Vaccine Construct for Licensing				
Interval between vaccination and challenge (days)	**Parameter**	**Effect of vaccination**	**Challenge type**	**Reference**
7	Clinical signs	Protected from disseminated disease and viremia, 1/5 had dental pad lesion	Intradermolingual injection	Pacheco et al., 2005
7	Clinical signs	Protected	Intradermolingual injection	Grubman et al., 2010
7	Clinical signs	Most cattle protected at 2 highest vaccine doses (dose response study)	Contact with infected cattle	Grubman et al., 2010
7	Virus transmission	No transmission between vaccinated cattle	Contact with infected cattle	Grubman et al., 2010
7	Virus transmission	Reduced or absent transmission to nonvaccinated cattle	Contact with infected cattle	Grubman et al., 2010

Table 6: Pigs – Various Experimental Vaccine Formulations					
Interval between vaccination and challenge (days)	**Parameter**	**Effect of vaccination**	**Vaccine Details**	**Challenge type**	**Reference**
7, 14 or 42	Clinical signs	Completely protected	Single dose of 5 x 10^9 pfu; vaccine without 2B	Injection	Moraes et al., 2002
7, 14 or 42	Virus shedding	Not detected in nasal swabs or blood	Single dose of 5 x 10^9 pfu; vaccine without 2B	Injection	Moraes et al., 2002
21	Clinical signs	Partially protected	Single dose of 5×10^9 pfu; vaccine without 2B	Injection (high dose/ severe challenge)	Pena et al., 2008
21	Virus shedding	Decreased virus shedding in nasal secretions; decreased viremia	Single dose of 5×10^9 pfu; vaccine without 2B	Injection (high dose/ severe challenge)	Pena et al., 2008
21	Clinical signs	Completely protected in one trial; partially protected in other trial	Single dose of 5×10^9 pfu; vaccine with 2B	Injection (high dose/ severe challenge)	Pena et al., 2008
21	Virus shedding	No virus shedding in nasal secretions or viremia in one trial; Decreased shedding and viremia in other trial	Single dose of 5×10^9 pfu; vaccine with 2B	Injection (high dose/ severe challenge)	Pena et al., 2008
56 (8 wks)	Clinical signs	Partially protected	Single dose of 1 x 10^8 pfu; vaccine without 2B	Injection	Mayr et al., 2001
56 (8 wks)	Clinical signs	Completely protected	2 doses of 1 x 10^8 pfu; vaccine without 2B	Injection	Mayr et al., 2001

12. INTERFERON AS A POTENTIAL EARLY PROTECTIVE MECHANISM

Summary
FMDV is very sensitive to interferon, and interferon-based protection might theoretically provide immunity before vaccine-induced immunity develops. Some studies have reported that pigs were protected from challenge after receiving conjugated interferon or hAd5-vectored constructs that express various interferons. High doses may result in side effects.

Other interventions might theoretically be combined with vaccination to provide immunity early, before vaccine-induced immunity develops [163]. FMDV is very sensitive to interferon (IFN) [10;329-331], and interferon-based protection has been evaluated in a number of studies, primarily in pigs. There is limited experience with conjugated interferons. In one experiment, pigs treated with different doses of conjugated recombinant porcine IFNγ (fused with glutathione S-transferase) were either completely or partially protected when challenged with FMDV 2 days later [332]. Transient fever was reported for 2-4 hours after the interferon injection.

Most studies have used hAd5-vectored interferon constructs, which have the advantage of prolonging the effect of the interferon and reducing the side effects associated with its administration [333]. In one experiment, pigs inoculated with both an hAd5-vectored FMD vaccine and an hAd5-vectored porcine interferon-α gene were completely protected from clinical signs, viremia or virus shedding, when challenged after 5 days [334]. Pigs that received the IFN construct alone were completely protected from viremia and clinical signs if they were challenged 1 or 3 days later, and partially protected if challenged 5 or 7 days after or 1 day before IFN administration [334]. Pigs that received the vaccine alone had less severe lesions than the controls, but the virus was shed in nasal secretions and one pig had low viremia [334]. The only side effect attributed to the interferon was elevated body temperature, lasting 1-2 days, in some animals. 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 [335]. Pigs receiving 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 [335]. 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 [336]. 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 [336].

While earlier studies all used FMDV serotype A_{24} Cruzeiro, a later study evaluated protection against two other serotypes. hAd5-vectored porcine IFN-α was shown to provide at least partial protection against FMDV A_{24} Cruzeiro, O_1 Manisa and Asia-1, when pigs were challenged 1 day after inoculation [337]. The response appeared to be dose-dependent, and complete protection was only observed with higher doses (10^{11} FFU/animal). However, higher doses caused jaundice and loss of appetite, and animals took 2-3 days to recover [337]. 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 [337].

There are a few reports of IFN administration in cattle. In one study, the clinical signs were less severe when the animals were given hAd5-vectored IFN-α; however, the animals were not completely protected [338]. 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. Unpublished studies in cattle at PIADC also reported limited protection, using hAd5-vectored IFN-α given alone or in combination with an FMD vaccine [10]. Whether the dose of the construct was too low to result in sustained IFN-α levels in this case, or other factors are involved is not known [10]. A later study examined the ability of hAd5-IFNλ3, alone or combined with hAd5-vectored porcine IFNα , to protect cattle challenged one day later with serotype A_{24} Cruzeiro viruses (by intradermolingual inoculation) or serotype O_1 Manisa viruses (in aerosols) [339]. 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 porcine IFNα construct alone seemed to have little or no effect.

Alphavirus-based empty replicon particles and/or porcine IFNα cloned into the VEE replicon vector appeared to be promising in FMDV-infected cells and mice [340], but have not been tested in livestock.

13. DURATION OF IMMUNITY

Summary

The OIE recommends that manufacturers of FMD vaccines evaluate the DOI by challenge or other validated tests, such as serology, at the end of the period of protection claimed on the label. Alternatively, they should indicate that the DOI is unknown. The OIE also recommends that the manufacturer demonstrate the efficacy of the recommended booster regimen.

There is limited information on the persistence of immunity after an animal recovers from FMD. Some studies suggest that immunity can last for at least 6 months to a year in cattle, with a few reports indicating that it might persist for as long as 4-5 years in some individual animals. Immunity to FMDV does not appear to last as long in pigs, and some animals become ill when re-challenged 3-6 months after infection.

Conventional inactivated FMD vaccines used for routine prophylaxis are expected to provide only 4–6 months of immunity; livestock immunized with these vaccines are typically re-vaccinated 1-3 times a year, depending on the species, its life expectancy and economic value, as well as the type and quality of the vaccine and the epidemiological situation. The first vaccination with aluminum hydroxide-adjuvanted vaccines may provide only 3-4 months of protection in cattle, with better immunity maintained after several inoculations. There are a few reports of vaccine-induced immunity lasting for a year or more in this species. In some cases, boosted conventional inactivated FMD vaccines may provide protection up to 6 months in pigs. There is relatively little information on the DOI for emergency (high potency) inactivated FMD vaccines, but a few studies suggest that these vaccines may protect cattle, sheep or pigs for 6-7 months. Some studies and authors suggest that oil adjuvanted vaccines have a longer DOI in ruminants than aqueous vaccines, while others feel that it might be similar. The DOI for the hAd5-vectored A_{24} Cruzeiro vaccine is currently unknown.

The duration of immunity (DOI) of a vaccine is an important consideration in vaccination-to-live programs [1]. Challenge studies provide the most definitive evidence for DOI, but very few of these studies have been published in any livestock species. The maintenance of titers to FMDV for prolonged periods is also suggestive, although it is not conclusive. The OIE recommends that manufacturers of FMD vaccines evaluate the DOI by challenge or other validated tests such as serology, at the end of the period of protection claimed on the label [16]. Alternatively, they should indicate that the DOI is unknown [16]. The OIE also recommends that the manufacturer demonstrate the efficacy of the recommended booster regimen [16].

13.1 Immunity After Infection

There is only limited information on how long immunity persists after an animal recovers from FMD. Some factors that may affect the DOI include the host species, individual animal variability and the virulence of the virus strain [19]. Some studies suggest that immunity can last for at least 6 months to a year in cattle ([19]; and [341] cited in [19]), and possibly longer in some individuals. 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 ([243] cited in [19]). One of 3 cattle at another laboratory was protected from challenge 4.5 years after infection ([341] cited in [19]). 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 [342].

Immunity to FMDV does not appear to last as long in pigs, possibly because persistent infections do not occur in this species [19]. 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) ([343] cited in [19]). Only one of the 5 pigs in this experiment became ill when challenged at 4 months [19]. Other studies in swine reported that approximately half of the animals developed clinical signs when they were re-challenged 3-6 months after infection ([344;345] cited in [19]).

Little is known about immunity to FMDV in sheep and goats, but 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) ([346] cited in [19]).

13.2 Immunity After Vaccination

Conventional inactivated FMD vaccines used for routine prophylaxis are expected to provide only 4–6 months of immunity [68]; livestock immunized with these vaccines are typically re-vaccinated 1-3 times a year, depending on the species, its life expectancy and economic value, as well as the type and quality of the vaccine and the epidemiological situation [16;22;328]. In endemic areas, the first vaccination with aluminum hydroxide-adjuvanted vaccines may provide only 3-4 months of immunity in cattle, with better immunity maintained after several inoculations [31]. There are a few reports of immunity lasting for a year or more in vaccinated ruminants. 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 ([347] cited in [19]). In the 1960s, field studies conducted during routine vaccination campaigns in the Netherlands suggested that antibody titers might be maintained for several years ([348] cited in [19;22]). 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 DOI. Significant antibody titers were also found among vaccinated cattle in France, 6 years after immunization ended ([349] cited in [19]). 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 [22].

There is relatively little information on the DOI for emergency (high potency) inactivated FMD vaccines; however, a few studies suggest that these vaccines may protect cattle, sheep or pigs for 6-7 months [68;202;206;295;328;350]. Cox et al. reported that pigs vaccinated with oil adjuvanted emergency FMD vaccines and challenged after 7 months were protected from clinical signs [328]. Some of these pigs also maintained high titers to FMDV for up to 7 months. In one of the two trials in this study, there was little decline in FMDV titers over 141 days; in the other trial, the titers gradually declined, and they were undetectable in 2 individual pigs after 71 or 169 days. Selman et al. also reported high titers in pigs for at least 6 months after a single dose of vaccine [350]. Cattle were protected from clinical signs when

challenged 6 months after vaccination with a single dose of a high potency, oil adjuvanted serotype A vaccine [295]. All cattle maintained high anti-FMDV titers throughout this study, although the titers declined slightly by the day of challenge. FMDV could not be isolated from some of these vaccinated cattle, but subclinical infections (defined as virus isolation and/or a fourfold rise in virus neutralization antibody titers after challenge) were reported in most. The effect of vaccination on virus shedding was not evaluated. Increasing the potency of this vaccine five-fold did not affect virus shedding or provide any other apparent benefit [295]. Follow-up unpublished studies on these cattle found that immunity in some animals waned after 6 months [154]. Cattle vaccinated with oil adjuvanted SAT vaccines also maintained high titers for at least six months ([202] reviewed in [68]). In contrast, Barnett et al. found that titers in cattle immunized with an oil adjuvanted serotype A_{24} Cruzeiro vaccine were decreasing by 43 days after vaccination ([206] reviewed in [68]).

No studies have been published, to date, in sheep challenged more than 28 days after a single dose of an emergency vaccine [68]. Sheep vaccinated with high potency emergency FMD vaccines maintained titers for up to 6 months [68;328;350]. Titers varied with the adjuvant and the specific antigen, and were maintained best with one oil adjuvanted (Montanide ISA 206) vaccine. Duration of immunity experiments have not been conducted with emergency FMD vaccines in goats. However, a group of goats vaccinated with conventional 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 [205].

Some studies and authors suggest that oil adjuvanted vaccines have a longer DOI in ruminants than aqueous vaccines [19;205]. Others feel that the DOI might be similar for the two adjuvants [19], based on certain laboratory studies and the prolonged serological responses during routine vaccination campaigns with aluminum hydroxide adjuvanted vaccines in the Netherlands [348] and France [349]. The DOI for the hAd5-vectored A_{24} Cruzeiro vaccine is currently unknown.

14. LIMITATIONS OF EXPERIMENTAL STUDIES

Extrapolation from experimental studies to the field situation must be done with care. For example, the reproduction ratio can be affected by the density of animals and their interactions, as well as the viral strain, the infectivity and susceptibility of individual animals, and the animals' species ([351] cited in [139]). The dose of FMDV and route of challenge may also differ between experiments and the field. Vaccine efficacy can vary due to concurrent diseases and other factors, and animals will be exposed to field viruses at different times after vaccination, rather than at a defined interval. Epidemics are also unpredictable, and experiments can never reproduce all possibilities.

15. FIELD EXPERIENCES WITH EMERGENCY FMD VACCINATION

Summary

Routine annual vaccination was a component of FMD control in Europe from the mid-20th century, when FMD was common, until 1991-1992. Together with other control measures, the European vaccination programs helped decrease the number of outbreaks, which suggests but does not prove that vaccination reduced transmission between farms. Some FMD-free countries throughout the world have used vaccination as a component of eradication programs, either with or without stamping out, when outbreaks occurred. Ring vaccination was used successfully during a localized outbreak in Albania in 1996, together with stamping out and the slaughter of infected animals. All susceptible species were vaccinated twice, at a four-week interval. Further disease transmission was not reported after the first round of vaccination, and eradication was completed within two months. Similarly, localized vaccination was part of an FMD eradication strategy during an outbreak in Macedonia (with virus spread stopped within three weeks) and in affected provinces of the Republic of Korea in 2002.

The Netherlands used vaccination as part of an eradication campaign in 2001. Vaccination was performed in an outbreak area with a dense livestock population, when there were insufficient resources for rapid pre-emptive culling and carcass disposal. Ring vaccination was used initially in limited areas, but more widespread vaccination was implemented when virus spread could not be controlled with these measures. Trade and agricultural organizations were consulted when choosing vaccination-to-kill or vaccination-to-live, and considerations involving international trade resulted in selecting vaccination-to-kill. However, the large scale destruction of apparently healthy, vaccinated animals was highly controversial among the public and some farmers. Since the outbreak, politicians in the Netherlands have been prominent in advocating changes in E.U. FMD legislation. After new E.U. FMD legislation and changes in the OIE Terrestrial Animal Health Code were implemented, vaccination-to-live with DIVA testing and the culling of infected herds became the preferred Dutch vaccination policy in an FMD outbreak.

Widespread vaccination campaigns to eradicate FMD were conducted in Uruguay and Argentina in 2000 and 2001. In Uruguay, a nationwide vaccination program, without stamping out, eradicated the virus from all species in approximately 4 months, although only cattle were vaccinated. While the number of infected farms was similar to the 2001 FMD outbreak in the U.K., the cost of eradication was considerably less in Uruguay, and far fewer animals were destroyed. Mass vaccination was combined with movement bans and other measures, including culling, at various times during the 2000-2002 outbreaks in Argentina. The eradication campaign in Argentina was complicated by the occurrence of several different FMDV isolates, and the necessity of adding two new strains to the vaccine over the course of the outbreaks. Mass vaccination and movement bans successfully decreased transmission in Argentina, but eradication took more than a year.

Vaccination campaigns were also conducted in Japan in 2010 and the Republic of Korea in 2010-2011. In both cases, the outbreaks were extensive, culling was delayed on some farms, and vaccination appeared to help stop further virus spread. Vaccination-to-kill was employed in Japan, and vaccination-to-live in Korea. Japan regained the status of FMD-free without vaccination after the outbreak, while Korea continued to vaccinate animals routinely and is classified as FMD-free with vaccination. The intensive strategy Japan used to document freedom from infection after the outbreak included random clinical assessment of cattle and swine, laboratory testing, the use of sentinel cattle, and sampling of susceptible wildlife.

An outbreak among pigs in Taipei, China (Taiwan) illustrates the difficulties in conducting a successful vaccination campaign when resources and advance planning for FMD control are inadequate. Despite these limitations, as well as inadequate movement controls and other difficulties, eradication was eventually successful. A relatively unpurified vaccine was used in this vaccination campaign, and the persistence of antibodies to FMDV SP and NSP proteins was later examined.

In Europe, annual vaccination was a component of FMD control beginning in the mid-20th century, when FMD was common, and lasting until 1991-1992, when routine vaccination was prohibited [68] Together with other control measures, the European vaccination programs helped decrease the number of outbreaks, which suggests that vaccination reduced transmission between farms [139]. However, it is difficult to compare this situation to limited emergency vaccination during an epidemic. Emergency vaccination has been a component of some recent eradication programs including limited outbreaks in Albania and Macedonia in 1996, the Republic of Korea in 2000, and the Netherlands in 2001; more extensive epizootics in Uruguay and Argentina in 2001; and an outbreak that affected only pigs in Taipei China in 2001. Vaccination was also used during outbreaks in Japan in 2010, and the Republic of Korea in 2010-2011. None of these countries conducted routine vaccination before the outbreaks, and all were FMD-free.

15.1 Albania, 1996

In 1996, an outbreak of FMD serotype A occurred in southeastern Albania [132]. Ten villages, all within a 15 km radius, were affected. Albanian authorities were assisted by a team of international experts from the E.U. and the European Commission for the Control of Foot and Mouth Disease (EUFMD). The control measures chosen included stamping out, with the slaughter of infected animals, and ring vaccination within and around the infected area. The emergency, monovalent vaccine was supplied by the E.U. and the Food and Agriculture Organization (FAO), and was available within 2 weeks of the decision to vaccinate. All susceptible species were vaccinated twice, at a four-week interval. Further disease transmission was not reported after the first round of vaccination, and eradication was completed within two months. No additional outbreaks were reported in the outbreak area or in unaffected villages where animals were vaccinated. Serological surveillance, conducted a year later, detected a few animals with NSP antibodies in a 3ABC ELISA. These animals, which had apparently been subclinically infected, were slaughtered. Serology also documented the absence of FMDV outside the initial area that had been affected. Albania reported the absence of FMD to the OIE between 1999 and 2008, with the last reported outbreak date of June 1996 [277;352], and it is currently recognized as FMD-free where vaccination is not practiced [353].

15.2 Macedonia, 1996

In 1996, the same strain of FMDV also affected the former Yugoslav Republic of Macedonia [132]. The first outbreaks occurred at Aracinovo, with virus spreading to the Skopje area. In total, 18 villages were affected. Stamping out was used in conjunction with vaccination. Approximately 4,500 animals were culled and all cattle in the area were immunized with a vaccine supplied by the E.U. and FAO. These measures stopped the spread of the outbreak in less than three weeks. FMD has not been reported in Macedonia since July 1996 [277;352], and it is currently recognized as FMD-free where vaccination is not practiced [353].

15.3 Republic of Korea (South Korea), 2000

In March 2000, serotype O (Pan-Asia lineage) FMDV was reported on a dairy farm in the Kyonggi province in the Republic of Korea [354]. In addition to stamping out, movement controls were placed on all animals and animal products within a 20 km radius of the infected farm. Differential restrictions were placed in the protection zone, which was defined as the area within a 10 km radius, and the surveillance zone, which extended to a 20 km radius from the farm. Two FMD outbreaks were reported approximately 150 km south of the index case in March, and another infected farm was detected approximately 140 km southwest on April 15. Approximately 2,200 cattle had been slaughtered by the end of April.

In mid-April, a decision was made to incorporate vaccination-to-kill in the affected provinces. At the time, the use of vaccination-to-live required a waiting period of 12 months (reduced to 6 months in 2002) after the last case or last vaccination, before a country could regain FMD-free status. The waiting time after vaccination-to-kill was only 3 months after vaccinated animals were slaughtered. National Veterinary Research and Quarantine Service and provincial veterinary officers immunized all cloven-hooved livestock in the three affected provinces with a monovalent, oil adjuvanted vaccine (strain O_1 Manisa) by August 2000. Approximately 860,000 animals were vaccinated during the first round, and approximately 662,000 animals received a booster. Vaccinated animals were marked, registered and placed under movement restrictions [154]. They were slaughtered either in a government indemnity program or they were sent by farmers to designated abattoirs [354]. Nearly 200,000 animals were slaughtered between the two rounds of vaccination, and approximately 563,000 had been culled by the end of April 2001. Surveillance programs, using clinical signs and serological assays, did not identify any additional infected animals after April 16, 2000 within the vaccination zones, and serological surveillance did not detect any cases in the rest of the country. During the recovery phase, surveillance by NSP ELISA exceeded OIE requirements, and was estimated to detect approximately 0.25% interherd prevalence (0.63% intraherd prevalence), assuming a test sensitivity of 95% at a confidence level of 95% [154] Only

dairy cattle and Korean native yellow cattle were affected in this outbreak [354]. No infected pigs were found, although they were highly susceptible to experimental infection with the outbreak strain. It is possible that management practices helped limit the spread of the virus between pigs and cattle. In addition, all swine herds in the protection zones were culled rapidly. South Korea became FMD-free without vaccination in September 2001 [355].

15.4 The Netherlands, 2001

The Netherlands used vaccination as part of an eradication campaign in 2001, when FMDV spread from the epizootic in the U.K. A ban on animal movements had been in place in the Netherlands since March 13, when FMD was reported in France [4]. Two infected farms, a mixed dairy goat/ veal calf farm and a dairy cattle farm 5 km away, were identified in mid-March [356]. These farms were in an area with a relatively dense livestock population, and approximately 3 weeks were thought to have passed between the most probable introduction of the virus and recognition of the outbreak. Protection zones with a radius of 3 km and surveillance zones of 10 km were placed around the infected farms [4;356]. Initially, infected herds, contact herds, and all herds on farms within a 1 km radius were preemptively culled. However, the resources to preemptively cull animals and quickly dispose of the carcasses were judged to be inadequate. For this reason, vaccination-to-kill was implemented in a 2 km radius around infected farms after March 28.

By April 1, FMD had been reported on 12 premises and seemed to be spreading more widely than expected [4]. There were also difficulties in rapidly tracing virus transmission between farms. Two options were considered for a more widespread vaccination plan: 1) vaccination-to-kill in all species, or 2) vaccination-to-live of dairy cattle, with vaccination-to-kill of pigs, sheep and goats [4]. The second option required that vaccinated cattle remain within the vaccination zone for at least 12 months, although their meat could be traded after 30 days and processed milk could be sold. However, swine, sheep and goat farmers within the vaccination zone would not have access to E.U. markets for at least a year. In contrast, the European Commission would allow trade in animal products to continue within the E.U. one month after the last vaccinated animal was killed, if vaccinated cattle were not allowed to live. Trade and agricultural organizations recommended the latter option [4].

Emergency vaccination was conducted mainly in one area, where most of the outbreaks had occurred. The borders of the vaccination zone included a river, a forest and two railway lines [356]. Approximately 850 herds were vaccinated. Emergency vaccination was also conducted in 175 herds around an outbreak in Kootwijkerbroek [356]. Infected herds continued to be culled immediately after their detection, with pre-emptive culling of contact herds as soon as possible. The vaccination campaigns took 1-2 weeks in some areas, but up to 4 weeks in the two main vaccination areas. In the Kootwijkerbroek area, vaccination was slowed by protests from farmers against the eradication campaign. Most herds were slaughtered between 11 and 40 days after vaccination. On April 10 and 11, two infected farms were reported in the northern part of the country. These herds and all herds with susceptible animals within a 1-2 km radius were vaccinated, then culled; 34 herds were affected [356]. The last infected herd was reported on April 22. The last vaccinated animal was killed on May 25, and the Netherlands regained its FMD-free without vaccination status in August 2001 [4].

During this outbreak, 26 herds (dairy cattle, two dairy goat farms and one veal calf farm) were found to be infected with FMDV, and 2763 herds containing approximately 260,000 animals were slaughtered [356]. Approximately 177,000 animals in 1931 herds were vaccinated [356]. One estimate suggests that less than 10% of the animals killed were probably infected [3]. The large scale destruction of apparently healthy, vaccinated animals generated intense criticism in the Netherlands among the public, and resulted in considerable discussion both in the press and in Parliament [3;4]. Dairy farmers and hobby farmers, in particular, opposed the decision to kill vaccinated animals [4]. A number of lawsuits were initiated against the government, and newly-created local action groups called for an immediate halt to the

slaughter-only policy [3;4]. The Dutch farmers union also became a target of criticism from some of its members and the public [3]. This union later published a position paper which, among other proposals, supports the use of protective vaccination-to-live, and states that continuing a policy of slaughter only is unacceptable [3]. Since the outbreak, politicians in the Netherlands have been prominent in advocating changes in E.U. FMD legislation [4]. After new E.U. FMD legislation and changes in the OIE Terrestrial Animal Health Code were implemented, vaccination-to-live with DIVA testing and the culling of only infected herds became the preferred Dutch vaccination policy in an FMD outbreak [4].

15.5 South American Vaccination Campaigns

In South America, vaccination continued to be a part of FMD control until 1994-2000, when Uruguay, Argentina, Paraguay and parts of Brazil, which were designated "FMD free where vaccination is practiced" stopped vaccination in order to gain "FMD free without vaccination" status, which carries fewer trade restrictions [129]. FMDV entered Argentina, Brazil and Uruguay from neighboring endemic regions in 2000 and 2001, resulting in widespread outbreaks in the region.

15.5.1 Uruguay, 2001

In Uruguay, a serotype O outbreak, which infected a single farm on the border with Brazil, was successfully controlled by stamping out in 2000 [129]. Approximately 12,400 sheep, 6,900 cattle and 257 pigs were destroyed, and control measures were completed with a month [129].

The initial control strategy during an outbreak with a serotype A virus in 2001 consisted of movement restrictions, ring vaccination and the culling of infected herds [129]. The first infected farm was reported on April 23, 2001, near the border with Argentina. Thirty-nine of the 430 cattle on the affected farm had signs of FMD; however, lesions were not found on the farm's 640 sheep. The affected and exposed animals were killed the following day. On April 26, FMD was detected on a neighboring farm, which had a mixed population of cattle, sheep and pigs. At the same time, several infected premises were found 40 km from the first cases. Ring vaccination with a radius of 10 km was initiated around infected farms, to prevent the virus from spreading outside the outbreak area. However, animal movements had spread FMDV beyond this region before the disease was detected, and local farmers also strongly resisted the stamping out campaign. As a result, the stamping out campaign was suspended, and a nationwide vaccination program for cattle was initiated in May [129]. The vaccine was provided to farmers free of charge, and the farmers were responsible for vaccinating their animals within a given time period. Infected farms continued to be quarantined, although the animals were not culled. Between April 27 and June 7, animal movement was banned throughout the country, but movement restrictions were relaxed after the vaccination campaign.

Government-administered serological tests at the completion of the vaccination program suggested that compliance had been high. Uruguay's 12 million sheep, which share pastures with the cattle, as well as the approximately 270,000 pigs, were not vaccinated; however, this did not seem to hamper the eradication of the virus. Approximately 40-60 infected farms were found each day at the height of the outbreak, but by the end of the first round of vaccination, there were fewer than 10 new foci per day [129]. All cattle were revaccinated between June 15 and July 22. A few days after the completion of the second round of vaccination, only a few sporadic cases were being found, and the last case was detected on August 21. In November 2001, 4.5 million young cattle that had been born since 2000 were vaccinated or re-vaccinated. By October, Uruguay was again classified as "FMD-free where vaccination is practiced." It has continued routine FMD vaccination due to the continued risk of virus reintroduction from other countries in South America.

The direct cost of eradication in Uruguay was estimated to be $13.6 million in U.S. dollars, with vaccine purchases accounting for $7.5 million of that total [129]. Estimated losses from the closing of external markets to Uruguayan farmers exceeded $200 million. Losses associated with closed packing plants, as well as the return of 380 containers of meat that were in transatlantic transit at the time of the outbreak,

added approximately $30 million. In total, the epidemic cost Uruguay approximately $244 million, and approximately 6,900 animals were culled during the early stages before vaccination was begun. In contrast, the 2001 epizootic in the U.K., where stamping out included the depopulation of all susceptible livestock within 3 km of an infected premises, cost an estimated $10 billion in losses to agriculture and tourism [357]. It also resulted in the culling of at least 4 million and possibly as many as 10 million animals (DEFRA statistics suggest that the true number is probably closer to 6 million) [358]. The number of infected animals in these two outbreaks was similar: the total number of confirmed foci in the U.K. was 2,030, while 2,057 farms or facilities were affected by FMD in Uruguay [129;358].

15.5.2 Argentina, 2000-2002

Argentina also used vaccination as part of its eradication campaigns in 2000-2002, but the form of the eradication program varied over the course of the outbreak. Several different viruses were involved. The majority of the cases were caused by serotype A viruses, with two different strains responsible for outbreaks from 2000 through early 2001 (A Argentina/2000) and outbreaks in 2001-2002 (A Argentina/2001); however, serotype O viruses were also detected in 2000 [359;360]. The initial control efforts in 2000 included movement restrictions throughout the country and the vaccination of contact herds with a bivalent serotype O and serotype A vaccine (O_1 Campos and A_{24} Cruzeiro), as well as culling [359]. After September 17, movement restrictions were applied only to infected and contact herds [359]. Approximately 270 serotype A outbreaks occurred during the first two months of 2001, although they were not officially reported internationally and have not been described [360]. They were controlled by ring vaccination, movement restrictions on infected and contact herds, and in some cases, culling of the infected herd. A Argentina/2000 was added to the vaccine in January 2001.

Outbreaks with a different serotype A virus were reported beginning in March 2001 [359;360]. During the earliest stages, the median time between the initial clinical signs in an infected herd and its recognition as FMD-suspect was estimated to be 13 days [360]. This factor, together with the high density of herds in the outbreak area, probably helped contribute to the magnitude of the epizootic: in total, approximately 2100 herds were affected with the new strain in 2001. The initial control strategies included ring vaccination around an infected herd, conducted twice at an interval of 3 weeks, and movement restrictions on infected and contact herds. A nationwide movement ban was also implemented between March 13 and April 3 [359].

Mass vaccination, twice a year, was begun in April in the affected region [359;360]. This vaccine used was quadrivalent; for the first time, it included the new A Argentina/2001 strain as well as A Argentina/2000, A_{24} Cruzeiro and O_1 Campos. Cattle were vaccinated again in early 2002 [359]. At this time, control measures were also modified to include the slaughter of all affected and in-contact animals [360]. The median herd disease reproduction ratio (Rh) was estimated to be 2.4 initially, decreased to 1.2 during the first vaccination campaign, and was lower than 1 after vaccination [359]. The mean (median) within-herd transmission rate (β) from February to December 2001 was estimated to be 0.26 (0.06) for unvaccinated herds and 0.17 (0.04) for vaccinated herds [361].The greatest distance between herd outbreaks was not changed by vaccination, and the spatial distribution was also similar, suggesting that outbreaks after vaccination were not from the reintroduction of virus, but because some animals were not protected by the vaccine [359]. Herd outbreaks (113) were reported up to 6 months after the end of the first mass-vaccination campaign, but no infected herds were reported after the second round of mass vaccination.

The 2001 FMD outbreak in Argentina suggested that a combination of mass vaccination and movement restrictions might be effective in ending epidemics, but eradication may take more than a year [359]. A small number of carriers were probably present, but virus transmission did not seem to occur, as determined by serology and the use of sentinel nonvaccinated cattle or other susceptible species [130].

Argentina is currently divided into two zones: one is FMD free without vaccination, while the other is classified as FMD-free where vaccination is practiced [353].

15.6 Republic of Korea (South Korea), 2010-2011

A swine herd infected with serotype O was first detected in the Republic of Korea on November 23, 2010; however, the diagnosis was delayed for a week due to false negative results from a rapid pen-side test [362]. This virus probably entered the country between November 9 and 16. Stamping out was used initially, with immediate depopulation after confirmation of the infection, together with preemptive slaughter of all cloven-hooved animals on farms within a radius of 500 m [78]. Many farms were thought to have been infected before the outbreak was detected [363]. Emergency vaccination was initiated on cattle farms in the outbreak area on December 25, using an oil adjuvanted, inactivated O_1 Manisa vaccine (≥ 6 PD_{50}) [78;362]. However, the virus continued to spread into new areas. Delayed culling by some at-risk farms was considered to be a contributing factor to a spike in infections during this time [362]. Nationwide vaccination-to-live was implemented on January 13, 2011, and all cattle and pigs had received 2 vaccine doses by February 26, 2011 [362;363]. Under the conditions in Korea, the number of cattle with titers to SPs peaked 2 weeks after vaccination, and the number of seropositive pigs (which developed lower titers) after 4 weeks [177]. Other cloven-hooved species (e.g., goats, deer) were also vaccinated after March 3 [363].

After all target animals had been vaccinated at least once, only animals with clinical signs and others suspected to be infected (e.g., animals that had been raised in the same pen) were culled [78;363]. The number of infected cattle farms began to decrease 12 days after the first vaccinations, and infected pig farms 18 days after the first swine vaccination [362]. The last cases were reported on April 21, 2011. A total of 3748 farms had confirmed infections during the outbreak and approximately 3.5 million cattle, pigs, goats or deer were culled during control measures [78;362]. Subsequently, routine vaccination-to-live has been performed with a trivalent vaccine in all cloven-hooved animals. Cattle and deer are vaccinated biannually and goats annually, while sows receive at least 3 doses a year and piglets are usually vaccinated once [78;177]. In 2011-2012, >95% of cattle and 60-80% of pigs in Korea had post-vaccination titers [177]. As of 2015, the Republic of Korea is considered to be FMD-free where vaccination is practiced [353].

15.7 Japan, 2010

Serotype O FMDV (Mya-98 lineage within the Southeast Asia topotype) was detected in southern Japan in 2010, initially on a beef farm in Miyazaki prefecture [7;58;364]. This area is the primary livestock-producing area of Japan, and has a high density of animals [58]. The first case in a cow with oral ulcers was identified and reported by a private veterinarian on April 9; however, confirmation of FMD was not until April 20, as other infectious diseases were initially suspected by the local veterinary service [58;364]. Epidemiological analysis suggests that the infection may have been introduced from a nearby water buffalo farm, where animals with nonspecific illness (fever, diarrhea) on March 31 were later found to have been infected with FMDV [58;364].

Infected farms were first reported outside the area of movement zone restrictions on April 28, with an increase to 10 newly identified farms per day by mid-May [58]. Infected pig farms were thought to have contributed significantly to virus spread, together with the local movement of people and vehicles in the infected areas [58]. As a result of rapid disease spread, culling could not be carried out immediately on newly identified premises [58]. While 88% of farms were culled within 1-2 days, the remainder required 3-10 days [154]. As a result, the decision was made to begin emergency vaccination on cattle and swine farms in the main region affected [58]. All FMD-susceptible animals on farms in the vaccination zone, including cattle, sheep, goats, swine, buffalo, deer and wild boar, were vaccinated [364]. Emergency vaccination was conducted in infected regions on May 22-26, using 126,000 doses of stockpiled O_1 Manisa vaccine [58]. Ring vaccination was carried out in a radius of 10 km around infected farms that

had been found as of May 18 (approximately 1,000 farms) [58]. The number of infected farms was appreciably decreased 2 weeks later [58], although some authors have noted that vaccination could have masked some cases [364]. The transmission parameter R became less than 1 at this time, and subsequently showed small fluctuations around 1 [364]. In this outbreak, 20 pig farms and 48 cattle farms were found to be infected 1-20 days after vaccination, with a major peak around 4-5 days after vaccination in cattle [58]. The median time between vaccination and the detection of these infected farms was 7 days for cattle and 9 days for pigs [58]. Some infected farms were still found outside the vaccination zone; however, the culling of these farms was completed within 3 days of detection, halting further transmission [58]. No further infections were reported after July 4 [58]. Transportation of vaccinated animals was prohibited, and all vaccinated animals had been culled by June 30 [58]. FMDV was found on 292 infected farms during the 3-month outbreak, and approximately 290,000 animals were destroyed in control efforts [7;58].

In addition to culling all vaccinated animals, the intensive strategy Japan used to document freedom from infection included random clinical assessment of all cattle and swine in Miyazaki prefecture, using a sampling method that could detect one infected animal at 95% confidence for a within-herd prevalence of 10% on all farms within a 3-kilometre radius of an unvaccinated, infected farm, as well as a random survey of 150 cattle farms at a level that could detect one infected herd if the herd prevalence was at least 2% with 95% confidence [154]. Sentinel cattle were placed on 175 of the 292 farms that had been infected, and wildlife susceptible to FMD (wild deer and boar) were screened by convenience sampling and found to be seronegative [58;154].

15.8 Taipei, China, 1997 (Vaccination in Pigs)

In Taipei, China (Taiwan), a type O virus affected pigs in 1997, but did not affect other species of livestock. The first cases were reported on March 14, with 28 infected farms recognized by March 20 [365]. By March 27, more than 200 premises on the western coast, which had a high concentration of pig farms, were known to be involved. The initial control measures included the depopulation of infected farms, movement restrictions on affected farms, and the vaccination of high risk farms. Transport of susceptible animals and meat products to offshore islands and the three unaffected counties on the eastern coast of Taipei was also banned. However, livestock auction markets were not closed during the outbreak. The epidemic peaked during the week of April 11, when approximately 1,100 newly infected farms were reported. Three infected premises were recognized in the eastern counties on April 21, April 23 and May 3. A protection zone with a 3 km radius and a surveillance zone extending to 6 km were placed around these three farms.

Plans to vaccinate all cloven-hooved animals on the island were initiated in late April, but there was a shortage of vaccines during the first month. Although the country had 10 million animals, only 40,000 doses were available, and plans to purchase additional vaccines were hampered by controversies over the vaccine type and source [366]. During the first stage of the vaccination campaign, there was not enough vaccine to immunize all of the animals on most farms, and priority was given to vaccinating animals in the surveillance zones [365]. Once additional vaccines became available between late April and early May, animals were immunized in the protection zones, beginning with sows and piglets, followed by fattening pigs, weaning pigs and susceptible animals of other species. After all pigs had been immunized at least once, the control policy was changed and only symptomatic pigs were destroyed; all pigs on infected farms were not culled. All cloven-hooved animals were revaccinated by early May. The epidemic declined significantly after the April peak, with an especially marked decline after the second round of vaccination. The last case was reported on July 15. During the four months of the outbreak, 6147 premises containing more than 4 million pigs were infected. The vast majority of cases were reported in the western regions; only 3 of 1398 swine farms on the eastern coast were affected. Offshore islands remained free of FMD.

In Taipei, the resources were inadequate for the size of the outbreak, especially during the first month [365]. The eradication campaign was unable to keep up with the number of new outbreaks until mid-May, after additional personnel had been recruited from the military. Many infected farms could not be slaughtered for 1-4 weeks after they were recognized, due to the lack of personnel, equipment and disposal facilities. Many farms were only partially vaccinated during the vaccine shortage, and many of these farms became infected. Movement controls were also inadequate. In particular, the livestock auction markets were not closed during the outbreak. During this outbreak, approximately 4 million pigs were destroyed, mainly in eradication efforts, although 4-5% (especially young piglets) died of the illness [365]. The vaccine cost $13.6 million in U.S. dollars. Approximately $187 million was spent, in total, on eradication activities, and economic losses to the pig industry were estimated to be $125 million. Major concerns by the general public included the safety of pork from infected animals, which led to a decline in pork consumption, and the ethics of culling uninfected animals. Pork consumption returned to normal by 5 months after the beginning of the outbreak.

A relatively unpurified vaccine, which induced NSP antibodies in some pigs, was used in this vaccination campaign [322]. Neutralizing antibodies to FMDV structural proteins remained high and persisted for more than 6 months in vaccinated pigs [322]. In contrast, NSP antibodies declined in most pigs over the first few months, and only small number of pigs had persistent NSP antibodies 6-12 months after vaccination. There was no evidence of virus circulation in these animals. Vaccinated pigs gave birth to some piglets with maternal antibodies to both structural proteins and NSPs.

16. STRATEGIES FOR VACCINE USE

16.1 Vaccination-to-Live and Vaccination-to-Slaughter

In an eradication program, animals may be either "vaccinated to live" or "vaccinated to slaughter." Animals that are "vaccinated to live" are allowed to live their normal lifespan unless they become infected. In contrast, animals that are "vaccinated to slaughter" are either slaughtered for human food consumption or killed and disposed of by some method. Both types of vaccination decrease the short-term resources required for carcass disposal, but will require the resources to implement, manage and maintain a vaccination, movement and permitting system for the vaccinates. Both types of vaccination are also expected to suppress virus transmission. Vaccination-to-live could potentially decrease the number of animals that must be culled. All other factors being equal, vaccination-to-live would result in the most benefits for animal survival and domestic continuity of business. However, the detrimental effect on exports is likely to be greater: countries must wait longer to apply for FMD-free status after emergency vaccination-to-live than vaccination-to-slaughter [70].

16.2 Approaches to the Application of FMD Vaccination

16.2.1 Prophylactic Vaccination

Prophylactic (routine) vaccination is generally used only in endemic areas or regions at high risk for FMD introduction, because it is a significant trade barrier for countries exporting animal products. The vaccine is chosen to target the serotype(s) and topotype(s) and/or lineages expected to be of concern in the region. Multivalent vaccines are often used.

16.2.2 Emergency Vaccination

Emergency vaccination (vaccination in the face of an outbreak) is usually conducted as reactive vaccination to a known strain of virus. This simplifies the choice of vaccine.

16.2.3 Protective Emergency Vaccination

Protective emergency vaccination, which is conducted among animals in uninfected areas, creates a zone of animals with reduced susceptibility around the infected area.

16.2.4 Suppressive (or "Damping Down") Emergency Vaccination

Suppressive (or 'damping down') emergency vaccination is conducted in the infected area where the virus is already circulating. It is intended to reduce virus transmission, aid control efforts and prevent FMD from spreading beyond the infected zone. For example, the Netherlands used suppressive vaccination to prevent FMDV from spreading when suspect farms could not be culled rapidly. Suppressive vaccination is likely to face a more severe virus challenge than protective vaccination: Infected animals may already be present on a farm in areas where this form of vaccination is used. In contrast, animals in uninfected areas (protective vaccination) are likely to be exposed to smaller amounts of virus in aerosols and on fomites.

16.2.5 Targeted Vaccination

Targeted vaccination attempts to protect specific groups of animals. Stamping out, as the sole eradication strategy, risks the destruction of rare species, rare breeds and high value genetic stock [3]. Targeted vaccination may be directed at uninfected animals of high value, which can include livestock with particularly valuable, rare or unusual genetic backgrounds, long-lived production animals, zoo animals or endangered species. Targeted vaccination can also be directed at uninfected areas where there is a high density of susceptible animals.

16.2.6 Ring Vaccination

Ring vaccination refers to a strategy of immunizing animals within a defined area around infected premises or infected zones. Its purpose is to reduce or prevent virus transmission from a focal outbreak to surrounding uninfected areas. Ring vaccination is most likely to be successful if foci of infection can be identified rapidly, before the virus can spread. It may not be appropriate in cases where the disease is widespread or contained in widely scattered foci, if the disease is difficult to identify, where there is a significant delay between infectivity and case confirmation, or where there is a significant delay between vaccine administration and the onset of protection.

16.2.7 Barrier Vaccination

Barrier vaccination is very similar in principle to ring vaccination; however, the vaccination zone is used to prevent the infection from spreading from a neighboring country or region into the uninfected area, rather than to keep it from spreading outward from infected premises. Geographic and political features usually have an important influence on the shape and location of the vaccination zone. Barrier vaccination can be used in an OIE-defined **protection zone,** in addition to enhanced surveillance and movement controls.

16.2.8 Predictive Vaccination

Predictive vaccination is a form of prioritization suggested by a model published in Nature in 2003 [367]. In predictive vaccination, vaccination is concentrated on farms that are predicted to have the greatest contribution to virus transmission in the future. Models are used to predict the probability that 'first generation' farms will be infected from a given source farm, and the probability that these farms will infect 'second generation' farms. Because first generation farms are likely to be infected before vaccination can take effect, the model suggests that they are not the optimal targets; vaccination should concentrate on farms at risk in the second generation. Although this model was developed based on data from experiences in the U.K., the principles are intriguing and might be applicable to other countries.

16.2.9 Blanket Vaccination

Blanket (mass) vaccination can be conducted throughout an entire country or throughout an OIE-defined zone with a separate status. Countries are most likely to consider blanket vaccination when a disease becomes widespread. This form of vaccination can be carried out indefinitely in countries or zones defined as "FMD free with vaccination"; however, this designation affects trade status.

16.3. Establishing a Vaccination Zone

Because surveillance must be conducted in vaccinated animals to identify acutely infected animals and carriers, and to demonstrate the absence of virus transmission after the outbreak, the vaccination zone should be the smallest area necessary to control the outbreak [178]. Factors to consider in establishing a vaccination zone include geographical barriers that limit disease spread, climatic conditions that could influence transmission (the greatest risk is close to the focus of infection), and the number of FMD cases and their distribution, as well as how long FMDV has probably been present, and its estimated future spread [15;178]. The numbers, density and species of animals in the vaccination zone, as well as the type(s) of husbandry and biosecurity practices must also be considered [15;178]. Defining the size and shape of a vaccination zone in ring vaccination can be complex. Modeling software may be able to estimate likely transmission zones for some pathogens and situations [15;184].

Consideration should be given to establishing a vaccination surveillance zone around the vaccination zone. In the E.U., the FMD Directive mandates a vaccination surveillance zone of at least 10 km diameter around the vaccination zone [178]. In the vaccination surveillance zone, no vaccination is allowed, movements are restricted and there is enhanced disease surveillance [178].

In the U.S.:

- The **Containment Vaccination Zone** is an emergency vaccination zone in the FMD Control Area. Vaccination may be performed in the Infected Zone and/or the Buffer Zone.
- The **Protection Vaccination Zone** is an emergency vaccination zone in the FMD-free Area. Barrier vaccination is used in this zone to prevent FMDV from spreading into areas free of the virus.

More information on each of these strategies can be found in the APHIS Framework for Foreign Animal Disease Preparedness and Response Plan (FAD PReP), USDA-APHIS.

17. MODELING STUDIES AND VACCINATION

Summary

Models have limitations, but they may provide insights into the possible impacts of vaccination approaches in specific scenarios. Some models based on the 2001 outbreak in the U.K examined optimal vaccination strategies when resources are limited, and may be of interest. Models suggest that the most advantageous control strategy may differ with the outbreak conditions and the priorities of the eradication program, and that early vaccination produces the greatest benefits, if vaccination will be used. Models also indicate that vaccination is not always economically beneficial even if it results in a shorter or smaller outbreak. Different models sometimes differ in their conclusions for a given set of data.

Few modeling studies have been published specifically for the U.S. One model, used to examine regional control strategies in an area of California that has a concentration of large-scale dairy operations, reported that vaccination was optimally targeted at these herds. Likewise, a study of feedlots in the Midwest suggested that targeting the largest feedlots (which have the greatest number of indirect contacts) produced the greatest benefits. Some other studies also suggest that vaccination could be a useful component of some eradication programs in the U.S., but the benefits of various control programs are likely to vary with the type of outbreak and the agricultural demographics in the region.

Models may provide insights into the possible impacts of vaccination approaches in specific scenarios. However, it should be kept in mind that outbreaks are unpredictable; models are a simplistic representation of the real world and incorporate subjective decisions on how to represent aspects of disease epidemiology; disease parameters must be estimated (and may be unknown or unmeasureable);

complex human value judgments are difficult to simulate; and there are always uncertainties in the model's assumptions [11;368;369]. Models also require reliable data [369]. While they may still be useful in generating hypotheses if they are based on theoretical or incomplete information, their limitations in this case must be disclosed, and the hypotheses will need to be investigated further [369]. The use of models as tools to predict the course of an ongoing outbreak is controversial. During the 2001 epizootic in the U.K., different models sometimes gave different conclusions, and some models were notably inaccurate in predicting the course of the epidemic [11;85]. One analysis of a model used in the U.K. suggests that, although models can predict the short-term future with very limited accuracy because virus transmission in the real world involves random elements, they become more accurate when considering the entire epidemic, where determining risk factors becomes more important [368]. Some sources promote the use of models as predictive tools to guide policies during epidemics, though some also note they should be used in conjunction with input from other sources such as field studies, laboratory studies and past experience. Others suggest that models are best used with real data to analyze hypothetical scenarios and intervention scenarios for past epidemics, as an aid in understanding the effects of various control measures, rather than as predictive tools [370].

Some modeling studies, based on conditions in various countries, have predicted benefits from vaccination (e.g., shorter or smaller outbreaks) exceeding the benefits from stamping out alone or preemptive culling [371-379]. Some studies also indicate that the initial conditions or outbreak situation may affect the relative benefits of the various control methods [371;379;380]. These conditions may have complexities beyond the density of the livestock population in the outbreak area. Models also suggest that the optimal strategy can be affected by the priorities of the control program (e.g., faster eradication, fewer animals culled, more rapid return to FMD-free status, lowest economic costs overall, reduction of the likelihood of a large epidemic, reduction of the size of the most likely epidemic), and a "one size fits all" approach may not be possible [373;375-377]. Many modeling studies have suggested that if vaccination is implemented, it is most effective started early in the outbreak [2;371;372;374;375], although one study of an outbreak among feedlots in the central U.S. suggested that vaccination decisions might not have to be made at the beginning of the outbreak [378].

Different models can sometimes give different conclusions about optimal control strategies in some circumstances, or they may predict outbreaks of different sizes [11;85;372;381]. For example, different outcomes were produced by 3 models (the North American Animal Disease Spread Model and models from Australia and New Zealand), using various outbreak simulations with data from the Republic of Ireland [372]. In this exercise, the North American model consistently predicted larger outbreaks than the other 2 models, in all control scenarios, and also predicted larger effects of interventions added to standard stamping out measures (i.e., a 33% reduction in infected premises for a contiguous cull, compared to 2% or 8% predicted by the other models, and a 50% reduction in infected premises for early vaccination compared to 7% or 10%) [372].

Two models examined optimal vaccination strategies when resources are limited. One study, published in Nature in 2003, modeled the efficacy of various forms of vaccination during a disseminated FMD outbreak in the U.K. such as the 2001 epizootic [367]. The model suggests that mass reactive vaccination, combined with culling of infected premises and epidemiologically identified "at risk" farms (i.e., "dangerous contacts"), might be effective if there are adequate resources. The assumptions are that vaccination would begin within a week, starting with the largest cattle farms, and reach a substantial proportion of the cattle within a few weeks after vaccination begins. In this model, vaccination can reduce the average size of the epidemic (which decreases rapidly as the daily vaccination rate increases) and also shortens the epidemic if high levels of herd immunity can eventually be established. If mass reactive vaccination is difficult to implement, the model suggests that predictive vaccination targeting high risk premises may also shorten the epidemic, although it has little benefit in reducing the size of the epidemic

[367]. In this form of vaccination, efforts are concentrated on the farms that are predicted to have the greatest contribution to the transmission of the virus in the future (i.e., the "second generation" farms described in section 16.2.8). This model suggests that ring vaccination has much less effect on the length of a highly disseminated outbreak [367]. Under its parameters, ring vaccination combined with culling is expected to decrease the epidemic size by only 20%, if the ring is 10 km and the efficacy of vaccination is 90%. Reasons for the limited effect of ring vaccination might include the time necessary for animals to develop immunity after vaccination, the transmission of virus from infected animals before they are recognized and reported, and the susceptibility of animals in neighboring nonvaccinated regions, which can be infected by long-range 'sparks' of infection. In addition, culling at-risk farms can remove potentially infected premises faster, which decreases the efficacy of ring vaccination when these two methods are combined. It should be noted that the results from this model contradict results from ring vaccination in other models [367;373;382]. Another modeling study based on the FMD outbreak in the UK suggests that when vaccination capacity is limited and reactive ring vaccination is combined with culling, it may be optimal to ignore farms that are likely to become infected before they are protected by the vaccine [368]. This model finds that prioritizing the largest farms first increases the optimal ring size and decreases the epidemic impact.

Few modeling studies of FMD vaccination have been published for the U.S. In addition to other uncertainties, modeling outbreaks in the U.S. faces difficulties in establishing the locations and sizes of agricultural establishments, and estimating the parameters governing transmission between premises [379]. Model validation is also difficult when there have been no recent outbreaks in a country, as the experiences in a past epidemic cannot necessarily supply information for a future outbreak [372]. (NB: Validation evaluates the confidence in the ability of the model to adequately describe disease outbreaks in the real world [369;372]. While a model is said to be internally valid if its outputs make epidemiological sense, it is externally valid only if its outputs are consistent with real epidemics [372].) One model, used to examine regional control strategies in an area of California that has a concentration of large-scale dairy operations, reported that vaccination was optimally targeted at these herds [383]. Large dairy herds have a high potential to transmit the virus because animals, people and vehicles frequently enter and leave the operations. They also have high value, with depopulation resulting in significant economic losses. This model found that vaccination had a relatively small impact in this region (a savings of 3-7%), and cost savings was important only if the epizootic spread quickly [383]. A model that examined an FMD outbreak in the central U.S., with virus introduction in a large feedlot, similarly suggested that vaccination may be best prioritized to large feedlots that have large numbers of indirect contacts [378]. While all vaccination-to-live scenarios in this simulation decreased the number of animals culled, with the greatest impact when the ring vaccination zone was larger, they did not all reduce the length of the outbreak. The scenario without vaccination resulted in rapid disease spread and also exceeded the depopulation capacity, while scenarios with vaccination did not. Other studies, based on expert opinions rather than modeling, indicate that is may be difficult to rapidly depopulate a large feedlot by humane means [384]. Vaccination to slaughter was not considered in this model; however, the authors noted that large feedlots are a natural target population for such programs, which might even allow for the use of these animals as food by controlled slaughter programs, reducing depopulation costs and the loss of valuable food (although it could extend the time until FMD-free status is restored). An economic analysis, published separately, suggested that vaccination in this situation would be beneficial in terms of epidemic costs ([385] cited in [378]). Some other modeling studies for the U.S. also suggested that vaccination could be a useful component of some eradication programs, but the benefits of various control programs are likely to vary with the type of outbreak and the agricultural demographics in the region, and that vaccination was not necessarily economically beneficial even if it resulted in a shorter or smaller outbreak [376;377;379].

18. MOVEMENT RESTRICTIONS AND VACCINATION

Effectively implemented movement restrictions have a significant impact on the effectiveness of a vaccination program [68]. If animals are unlikely to be exposed directly to high doses of virus via close contact with infected animals, the premises are likely to experience a less severe challenge. In this situation, vaccination would need to protect animals mainly from lower doses of virus via indirect transmission in aerosols and on fomites. This would, ideally, be the case in protective vaccination, which is conducted in a zone containing uninfected animals. Movement controls also help control virus transmission in areas where the virus is circulating; however, some premises might already contain infected animals. For this reason, the vaccine may face a more severe test of its efficacy when used in suppressive vaccination.

Vaccination alone often cannot achieve the level of population immunity needed to interrupt transmission where FMD is endemic [386]. If vaccination is not combined with effective biosecurity and movement controls, the virus can continue to circulate or be re-introduced from pockets of infection. In some parts of the Middle East, large dairy herds are vaccinated every 10 weeks with seven strains of FMDV, but outbreaks are not reduced unless strict biosecurity is practiced to isolate the herd from other livestock [9].

19. SPECIES TO VACCINATE

Cattle are usually the most important maintenance hosts for FMD viruses [16], and most vaccination programs concentrate on this species. During successful eradication campaigns in South America, mass vaccination programs were conducted in cattle, but sheep and pigs were not immunized [129;359;360]. However, the species to vaccinate may vary with the tropism of the strain. In Taipei China (Taiwan), a type O virus affected only pigs in 1997 [322]. The importance of small ruminants might vary with the strain and/or epidemiological factors [47-49;99-101]. There is currently no consensus about whether small ruminants should be vaccinated during outbreaks [47;49;143]. Another consideration is the availability of NSP tests to detect infections in vaccinated animals. Validation of these tests in species other than cattle has been limited [42;143;151], although it is reported to be ongoing [70].

20. VACCINE SELECTION

The selection of a vaccine is based on many factors, including its degree of relatedness with the field strain (e.g., 'r' value), potency, availability in sufficient quantities from a reputable source, licensing considerations and other factors.

Conventional (standard potency/ prophylactic) vaccines, which are used routinely to control FMD in endemic areas, usually have a lower dose of antigen than emergency vaccines. The OIE recommends that these vaccines have a minimum potency of 3.0 PD_{50} per cattle dose for the duration of the shelf-life claimed by the manufacturer [16]. The advantage of conventional vaccines is that they may be immediately available. When they are used to vaccinate ruminants in endemic areas, conventional vaccines are usually boosted a few weeks later [68;328]. Animals are then revaccinated every 4–6 months to a year, depending on the species, its life expectancy and economic value, as well as the type and quality of the vaccine and the epidemiological situation [16;22;68]. With routine vaccination in endemic areas, the first vaccination with aluminum hydroxide-adjuvanted vaccines may provide only 3-4 months of immunity in cattle, with better immunity maintained after several inoculations [31]. In FMD-endemic areas, vaccinated animals usually have time to develop and maintain an adequate immune response before they are exposed [68].

In contrast, emergency vaccination may be followed very shortly by challenge [68]. For this reason, emergency vaccines usually have higher doses of antigen, to create immunity faster. A highly potent vaccine is more likely to protection against a heterologous virus than an equally cross-reactive but less potent vaccine, although it is possible that this effect does not occur with all strains

[16;109;210;223;279;284] Some well established vaccine strains such as A_{22} Iraq, O_1 Manisa, O_1 Lausanne, O_1 Campos and A_{24} Cruzeiro are very potent and protect against a range of field strains within the same serotype [1;19;22]. Boosters can also be used to increase the protection provided by the vaccine and the breadth of antigenic cover; however, this is slower than using a highly potent vaccine [16;284], and cannot overcome large differences [22]. Both conventional and emergency vaccines supplied by reputable manufacturers and banks usually have PD_{50} levels well over the minimum stipulated values [1;68;109]. In one experiment, a single dose of a high potency FMD vaccine (serotype A) induced high titers of antibodies to FMDV, and protected cattle from challenge for at least 6 months, suggesting that boosters may not be necessary if the vaccine potency is high enough and the vaccine is closely related to the field virus [295]. After 6 months, however, antibody levels declined in some animals [154]. Decisions on vaccine potency may be a compromise influenced by the cost of the vaccine and the number of doses that are needed.

An emergency FMD vaccine should also be sufficiently pure that it can be used with DIVA tests that detect antibodies to NSPs [70].

21. HERD COVERAGE

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 [22]. However, the required level of herd immunity varies with its size and the density of the susceptible population, as well as the species within the herd [70]. 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 [70].

22. VACCINE ADMINISTRATION

FMD vaccines are administered according to the label instructions. In endemic areas where small ruminants are included in vaccination campaigns, they typically receive ⅓ to ½ of the cattle dose, using aluminum hydroxide or oil adjuvanted, inactivated vaccines, respectively [22;47;145]. This results in a volume of approximately 1 ml for a typical 2-3 ml aqueous vaccine or 2 ml oil vaccine [145]. Experiments using one serotype O_1 Manisa oil vaccine confirmed that the antigen payload in half a cattle dose was sufficient for good immunity in sheep and goats [145;146;316].

Although needle inoculation is currently used to deliver all licensed FMD vaccines, intradermal needle-free devices are expected to be more efficient, and may maximize the number of vaccine doses that can be obtained from antigen concentrates [387]. Vaccines administered intradermally seem to be more immunogenic, probably due to the large numbers of dendritic cells in the skin ([388]; and [389;390] cited in [387]). Needle-free devices have not been validated with FMD vaccines, and very few published studies have explored the use of these devices. In early experiments, however, such devices appear to be promising. In one study, 1/4 dose of an aqueous inactivated FMD vaccine was as effective as a full dose, when both were administered intradermally to cattle with a needle-free, compressed gas vaccination system [387]. 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 [387]. 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 [391]. .In this experiment, 13 pigs vaccinated IM with a full vaccine dose were protected from generalized FMD after challenge, although two animals developed vesicles in the inoculated foot. Virus shedding was also reduced, but two pigs shed virus briefly and at low viral titers. One of 13 pigs vaccinated ID with a 1/10 dose developed generalized lesions and three pigs developed vesicles on the inoculated foot. Subclinical virus shedding was reported in one animal. In this experiment, a ten-fold higher antigen dose did not improve protection in the ID-inoculated pigs, possibly because the high viscosity of the preparation interfered with delivery using the needle-free device. With the higher

dose of antigen, one of 8 ID-inoculated pigs developed generalized FMD, five pigs had lesions on the inoculated foot, and one pig also had a fever. Subclinical virus shedding was reported in three pigs, although in two cases it was brief and at low titers. In contrast, pigs vaccinated IM with a five-fold higher dose were clinically protected from challenge and did not shed virus. Surprisingly, virus shedding in oropharyngeal samples was not detected in either ID-inoculated pig that developed generalized lesions. The authors speculate that mucosal immunity induced by this form of vaccination might be responsible for suppressing virus excretion. Localized reactions, with granulation tissue and necrosis, were observed at the inoculation site in both IM- and ID-inoculated pigs, and a 2-5 cm diameter swelling was seen at the ID inoculation sites. The use of transdermal injection with needle free devices is also being investigated for hAd5-vectored FMD vaccines, [253] but no information is currently available. Sublingual inoculation was reported to protect laboratory rodents with as little as 1/16 the normal FMD vaccine dose [392], but this method of delivery has not been tested in livestock.

23. MATERNAL ANTIBODIES

Maternal antibodies to FMDV may be detected in the offspring of vaccinated dams. In calves born to nonvaccinated cows, some inactivated FMD vaccines can be given as early as 1 week of age [16], although there is conflicting evidence on whether the response is as effective as in adult cattle [22]. Maternal antibodies can interfere with vaccination in this species unless the titer is less than 1:45; however, the calf can become infected if the titer of antibodies to FMDV is below 1:100 in the liquid phase blocking ELISA [71]. The influence of these antibodies can last for several months, and occasionally up to 5-6 months ([393] cited in [22]), especially if the dam has been immunized repeatedly [22]. Maternal antibodies do not usually persist longer than 6 months [70]. The OIE suggests using information provided by the vaccine manufacturer to minimize interference with maternal antibodies [16]. Because calves can receive different levels of antibodies in colostrum, one method of ensuring more uniform responses, in regions where herds are vaccinated regularly, is to give equal amounts of pooled colostrum, followed by vaccination at 4, 5 and 6 months of age [71].

Maternal antibodies may interfere less with oil adjuvanted vaccines than aluminum hydroxide adjuvanted vaccines in ruminants [394], although some authors feel the evidence is still inconclusive [22]. In one recent study, an oil adjuvanted vaccine induced serological responses in some calves with maternal antibodies, but the titers declined after 30 days [394]. Another study suggested that calves may sometimes respond to an inactivated vaccine containing a different strain within the same serotype, although the response is limited [395].

Young pigs develop poor immunity to FMD vaccines; they are usually protected by vaccinating the sow in endemic areas [46]. Breeding sows may be vaccinated twice a year or more frequently, during their pregnancy. In pigs, maternal antibodies interfere with vaccination before 8 weeks of age [19;46;396], and in some cases, for as long as 10-12 weeks [397]. The first FMD vaccines are usually given at 10-12 weeks and repeated in 2 weeks [46], although other schedules have also been reported to produce good results under some field conditions [398]. Antibodies to NSPs as well as neutralizing antibodies were detected in some piglets, when sows had been immunized with an unpurified FMD vaccine during an outbreak in Taiwan [322].

In kids born to goats immunized with commercial quadrivalent FMD vaccines, maternal antibodies persisted for up to 90 days after birth [205]. The titers were similar whether the adjuvant was oil or aluminum hydroxide. Titers for serotype O were maintained at protective levels for only a week after birth, but they remained at protective levels for serotypes A, C and Asia1 for up to 4 weeks. The authors suggest that kids should be vaccinated at approximately 3–4 months of age [205]. In lambs, protective levels of maternal antibodies are also thought to persist for up to 4 weeks of age [211]. Most young buffalo seem to become infected when they are approximately 2-6 months old, after maternal antibodies have decreased ([399] reviewed in [84]).

There is currently no information about the effectiveness of hAd5-vectored FMD vaccines in the presence of maternal antibodies to FMDV.

24. LIMITATIONS OF VACCINATION

Optimal protection of each individual animal is not usually possible during mass vaccination [185;324]. The level of immunity in each animal will be influenced by vaccine factors including potency, by the effectiveness of vaccine administration (e.g., the maintenance of an effective cold chain and proper administration), and by the closeness of the match between the vaccine and the field virus to which the animal is exposed. The animal's genetic background, immunosuppression (parasitism, poor nutrition, stress, etc.), an immature immune system in a young animal, advanced age and other factors that affect individual susceptibility will also affect the vaccine's efficacy. In vaccinated animals, immunity can be overwhelmed by a high challenge dose even if the vaccine is well-matched to the field strain. Animals may also be exposed before they have time to develop protective immunity.

24.1 Monitoring for Vaccination Coverage and Efficacy

Although other immune mechanisms may also protect animals from FMDV, in vivo protection correlates with titers of antibodies to FMDV ([219;282;283] reviewed in [284]). Acceptable tests to monitor the level of protection in vaccinated herds include the solid-phase competition ELISA, the liquid-phase blocking ELISA and VNT [16]. These tests all measure immunity to FMDV structural proteins. VNT titers that correlate with protection vary depending on the laboratory and test system and cannot be compared directly [284]. Some animals without titers at the time of challenge can be protected [207].

Serological tests can be used to determine vaccination coverage in eradication campaigns. Only single titers should be needed when animals have never been vaccinated against FMD outside an endemic region. Paired serum samples might be necessary after the first vaccination. Serological tests might also be useful in monitoring declining titers for the administration of boosters.

25. IDENTIFICATION OF VACCINATED ANIMALS

Vaccinated animals must be permanently identified, using an official NAFMDVB pink metal ear tag with individual identification. Accurate vaccination records must be maintained as directed by USDA APHIS VS, and shared with other regulatory authorities as required.

26. LOGISTICAL AND ECONOMIC CONSIDERATIONS IN THE DECISION TO VACCINATE

Summary

The technical feasibility of vaccination and funding for a vaccination campaign should be assessed before deciding to vaccinate. The assessment should include the availability of sufficient supplies of an effective, safe, well-matched and sufficiently potent vaccine; the availability of DIVA tests (if applicable); the logistics of vaccine administration; and the resources and technologies needed for associated activities including individual animal identification, traceability, movement permitting and serosurveillance to prove freedom from disease. The impact of vaccination, both positive and negative, on other eradication activities should also be determined.

The effectiveness and form of a vaccination campaign can vary with the epidemiology of the outbreak. Some factors that influence FMD epidemiology are the strain of FMDV (including its species specificity, virulence and ability to spread via aerosols), the length of time the virus has been present, geographic distribution of the virus, species of animals involved and herd size, geographic limits on virus transmission, and climatic factors that affect aerosol transmission. Wildlife and feral populations of domesticated animals may need to be considered in some outbreaks.

The pros and cons of vaccination compared to pre-emptive culling should be considered. Considerations

include the effects on trade and exports, market shocks, potential restrictions on marketing products from vaccinated animals, the types of stakeholders affected (e.g., small-scale operators with limited safety nets vs. large-scale operators), the extent of the outbreak and other factors such as the disruption of tourism or impacts on local economies.

Consideration should be given to whether genetically irreplaceable stock, endangered species or other unusually valuable animals can be successfully protected with biosecurity measures, and whether vaccination would be beneficial. Their degree of isolation from livestock should be part of this analysis.

Countries that eradicate FMD by stamping out, without using vaccination, can apply for OIE FMD-free status 3 months after the last case. If vaccination-to-kill is part of the eradication campaign, the country must wait until 3 months after all vaccinated animals have been slaughtered. If vaccination-to-live is used, the application can be made 6 months after the final case or the last vaccination, whichever occurred last. Serological surveillance must also demonstrate the absence of virus circulation in vaccinated herds that are allowed to live.

26.1 Technical Feasibility of Vaccination

To conduct an effective vaccination campaign, an effective, safe, well-matched and sufficiently potent vaccine must be available, and the vaccine supply must be sufficient to carry out the vaccination strategy in a timely manner. The vaccine and vaccination strategy should be expected to provide immunity quickly enough to stop or slow virus transmission. Consideration should also be given to whether animals would need to be vaccinated more than once (for increased potency or to protect animals from continued virus circulation), and whether the duration of immunity from the vaccine is acceptable. If there is only enough vaccine for the initial needs, the feasibility of procuring additional supplies should be determined before beginning the campaign. National contingency plans should include the predicted number of doses of emergency vaccine needed under the best, medium and worst case scenarios [1]. Before an outbreak, plans should also be made for vaccine distribution and administration, including the maintenance of an effective cold chain.

DIVA tests that can detect antibodies to NSPs must also be available, if vaccination-live is used. These tests must be validated for the species to be vaccinated. DIVA tests may not be a requirement in vaccination-to-kill unless the movement of these animals (except to slaughter under secure conditions) is allowed.

There must also be adequate numbers of trained personnel to conduct vaccination and other associated activities. If vaccination is used during an outbreak, fewer people and other resources may be available for procedures such as diagnosis, culling or decontamination of infected farms [10]. Conversely, vaccination may allow the culling of some animals to be delayed, and relieve pressures on personnel and resources involved in slaughter and disposal. Whether vaccination and identification of vaccinates by producers and private veterinarians would be permitted in the U.S. is uncertain; however, if allowed, this would increase the vaccination capacity considerably [378]. Finally, the resources and technologies needed for associated activities, including individual animal identification, traceability and movement permitting, must be available in a vaccination campaign.

26.2 Epidemiological Considerations

The effectiveness and form of a vaccination campaign can vary with the epidemiology of the outbreak, which may be influenced by the strain of virus, animal species, herd size and other factors. FMDV strains can differ in their virulence, species specificity and transmission in aerosols [11;15;42;178]. Aerosol transmission is also influenced by factors such as the prevailing winds and humidity, as well as transmission over water vs. land [11;85]. Geographic barriers such as mountains and deserts can limit the spread of the virus.

Consideration should be given to the length of time the disease has been present in the country, and how long it has probably spread undetected [178]. The age of the oldest lesions can help estimate when FMD was probably introduced. In cattle, a maximum of 14 days (the maximum incubation period) is added to the oldest lesions, while 11 days should be added in pigs [46]. The movements of livestock, people and vehicles should be assessed to estimate whether the virus has been spread widely from any premises known to be infected. A single focus or limited outbreak is expected to be easier to control than one that has become disseminated. Vaccination also tends to become more desirable as the size of an epidemic grows, because large epidemics face difficulties in the number of animals that must be culled, both in feasibility and in acceptability to stakeholders [176].

Although very few modeling studies have been published for the U.S., models may have the potential to help estimate the probable course of hypothetical outbreaks. However, they should be used with care, and their use as predictive tools during an outbreak is controversial. Exact premises and animal population data may improve the performance of some models.

Wildlife may also need to be considered in some outbreaks. FMDV does not seem to be maintained indefinitely in wild animals other than African buffalo, and eradication has been successful in countries that did not control the disease in wildlife [31]. However, the virus can infect some wildlife populations for a time [26;31;32;54-56], and they could transmit FMD to domesticated livestock if they come in contact. The potential to maintain FMDV in feral populations of domesticated animals, such as feral pigs, should also be considered.

26.3 Economic Viability of Vaccination

Economic viability plays an important role in the decision to vaccinate. There must be sufficient funding for the purchase of the vaccine, vaccine delivery and administration, and individual animal identification. In addition, funding must be provided for follow on traceability of the vaccinated animals and serosurveillance to prove freedom from disease.

The direct costs of vaccination include:

- Investment costs – e.g., vaccine development, vaccine availability and vaccine delivery infrastructure [400]
- Variable or recurrent costs including the cost of vaccines and delivery [400]
- Costs to identify vaccinated animals, permit their movement, and conduct surveillance to prove freedom from disease (in a vaccinate-to-live strategy)

There may also be some indirect costs from vaccination such as lost productivity caused by stress to animals, disruptions of agricultural routines, and adverse reactions to the vaccine [400].

The pros and cons of vaccination compared to pre-emptive culling should be considered. Culling herds that were never infected can cause economic losses without necessarily affecting disease spread. However, blanket vaccination or inappropriately targeted vaccination is expensive, and there is an increased risk that infected animals will not be detected because clinical signs are suppressed [2]. Vaccination-to-live programs, or vaccination-to-kill programs that later slaughter animals for food, can reduce the costs of depopulation, carcass disposal and food wastage; however, they may prolong the time until the country can be considered free from FMD for international trade.

The overall impact of vaccination on international trade in livestock products, including longer term impacts on trade, is an important consideration for FMD. Vaccination is expected to be most beneficial when the outbreak ends sooner, or when vaccination allows the most stringent disease control measures to be carried out in a limited area [400]. It is also expected to be beneficial if it impacts a livestock sector in

an area where there will be a limited effect on exports (e.g., zoning will be possible/practical). If the outbreak can be stopped with rapid culling, there is likely to be short-term distress but little long-term effect on livelihoods, especially if indemnity can be provided [400]. However, if culling is more widespread or the disease is out of control, vaccination may save livelihoods [400].

Vaccination is likely to be beneficial to livelihoods when it can:

- Provide effective disease control with little depopulation, especially if indemnity is not available for culled animals [400]
- Prevent national markets from being disrupted or rapidly restore them [400]
- Minimize other economically important factors such as the disruption of tourism or impacts on local economies [400]
- Reduce the time export markets are lost

Vaccination may be particularly beneficial to small-scale operators whose safety nets are limited [400]. If stamping out is used, it is possible for culling to have a minimal effect on the national economy while having a significant effect on the livelihoods of the people who are directly affected, especially smallholders and small-scale traders who depend on regular cash flow from agriculture. Although indemnity may be available for animals that must be destroyed, it rarely covers the cost of lost production time and cash flow [400]. The emotional impact of the destruction of apparently healthy animals should also be taken into consideration [400]. In the U.S., diseases have been controlled effectively in the past by culling infected and exposed animals, but there have been changes in agricultural practices, such as increased herd sizes, which may make the impact greater [199] and depopulation more difficult [384].

Consideration of market shocks should be part of the economic analysis. Market shocks can result from loss of consumer confidence (decreased demand), very severe culling or the closing of markets [400]. Unless consumers can be persuaded that products from vaccinated animals are safe, there may still be market shocks from consumer fear even if the disease itself is controlled by vaccination. Consideration should be given to whether meat, milk and other products from vaccinated animals can be used, and whether they will need to be treated (because vaccination might mask the presence of virus) before they are allowed into markets. If export markets are affected by vaccination, domestic markets can be affected, because animal products that were once exported may be sold within the country, lowering prices [400]. Producers for domestic markets can also be affected by quarantines. If animals are larger than normal weight and/or are released into the market in a short period after quarantine is lifted, prices may be lower [400]. The cost of keeping and feeding animals through the quarantine period should also be taken into consideration.

Modeling using the parameter "first-fortnight incidence" (FFI; the number of farms affected by FMD during the first two weeks) has been proposed as one way to help predict the prevalence and duration of an FMD outbreak, using data from its initial stages [2]. This model, which was tailored for the U.K., suggests that FFI is directly related to the final level of disease prevalence. FFI has been proposed as an estimate for whether vaccination-to-live would be economically viable [2]. If FFI suggests that the outbreak will last as long or longer than the length of the trade ban (the length of the epidemic plus the length of the post-epidemic export ban), this model suggests that vaccination should be considered early, either during or after the first fortnight. A recent modeling study, based on the livestock structure in Denmark, also suggested that FFI may be useful in predicting the duration, size and economic consequences of an outbreak in that country [401]. The first fortnight spatial spread (FFS; the circumference of the infected zone on the 14^{th} day after initial outbreak detection) was another predictor of the epidemic outcome in this study. However, it should be noted that it can be difficult to accurately predict the course of an outbreak, especially in the early stages when data may be limited or incomplete. Other characteristics of the known infected premises also need to be considered in early vaccination

decisions. Any country using a prediction model should tailor it to the specific conditions present there [401].

26.4 Vaccination of Genetically Irreplaceable Stock, Endangered Species or Other Unusually Valuable Animals

Consideration should be given to whether these animals can be successfully protected with biosecurity measures, and whether vaccination would be beneficial. Their degree of isolation from livestock should be part of this analysis.

26.5 Effect of Vaccination on Regaining OIE FMD-Free Status

Countries that eradicate FMD by stamping out, without using vaccination, can apply for OIE FMD-free status 3 months after the last case [70]. If vaccination-to-kill is part of the eradication campaign, the country must wait until 3 months after all vaccinated animals have been slaughtered. If vaccination-to-live is used, the application can be made 6 months after the final case or the last vaccination, whichever occurred last. Serological surveillance must also demonstrate the absence of virus circulation in vaccinated herds that are allowed to live. Evidence for the effectiveness of the vaccination program could be a valuable component of post-outbreak surveillance in this situation, even when it is not required by the OIE standards [176]. In addition to the cost-benefit analysis for vaccination, an analysis should be conducted to determine the economic impacts of vaccination-to-live compared to vaccination-to-kill for high-impact regions of the U.S. Recently, there has been some discussion about the disparity in waiting periods when vaccination is employed, with some authors arguing that it has no sound scientific basis [121;154]. The suggestion has been made that, instead, the OIE guidelines be revisited to focus on establishing acceptable levels of surveillance to substantiate the absence of FMDV infection or circulation, as a standard for resumption of trade [121;154].

The effects of immunization on the export of vaccinated animals should also be taken into consideration. Some trading partners may restrict the entry of livestock vaccinated for FMD. For example, an E.U. Directive states that animals vaccinated against FMD cannot be shipped between member states after a declaration of FMD freedom in a Member State [11].

27. VACCINATION IN ZOOS AND SPECIAL COLLECTIONS

Summary

There are special concerns about the potential loss of genetic diversity and risks to endangered species if FMD affects zoos, and animals become severely ill or must be culled to prevent virus transmission. High fatality rates have occasionally been reported in some species of wildlife or zoo animals. If even a single zoo were to be affected, a substantial proportion of the genetic diversity in some species could be lost. For these reasons, vaccination might be considered in exotic species when it is not a consideration in agricultural livestock.

Only killed vaccines should be used in zoo animals, unless the vaccine has been specifically tested in the target species. There are only a few reports of experiments on the use of FMD vaccines in zoo animals. Vaccination programs for FMD have been conducted in the past in zoos, and some limited information is also available from these programs. Whether the OIE guidelines for the use of FMD vaccines in domesticated livestock are appropriate for captive non-domesticated species is unknown.

There are special concerns about the potential loss of genetic diversity and risks to endangered species if FMD affects zoos, and animals become severely ill or must be culled to prevent virus transmission. If even a single zoo were to be affected, a substantial proportion of the genetic diversity in some species could be lost [32]. For these reasons, vaccination might be considered in exotic species when it is not a consideration in agricultural livestock.

Between 1950 and 1990, some European zoos regularly vaccinated susceptible species in their collections, similarly to programs in cattle [32]. These programs ended in 1991 when vaccination was prohibited in the E.U. Outbreaks were occasionally reported in European zoos before the area became FMD-free [32]. Amsterdam Zoo was affected during an outbreak among cattle in 1937. It was also infected repeatedly by FMD from 1950 to 1972. At least some of the latter outbreaks are suspected to have been linked to a nearby FMD vaccine plant. Vaccination was able to contain the outbreaks, and preventive vaccination was also carried out for part of this time. Another outbreak occurred at the Rotterdam Zoo in 1951. This virus was probably transmitted to the zoo when the head keeper of the yaks visited a farm with FMD, and illness was reported only in this species. All susceptible artiodactyls at the zoo were vaccinated during the outbreak. They included kudu, eland, nilgai (*Boselaphus trachocamelus*), Arabian oryx (*Oryx leucoryx*), gemsbok (*Oryx gazella*), blackbuck, Asian water buffalo, banteng (*Bos javanicus*), addax (*Addax nasomasculatus*), anoa (*Bubalus depressicornis*), wildebeest, American bison, wapiti (*Cervus canadensis*), pudu, fallow deer and babirusa. Some zoos outside the E.U. still vaccinate susceptible species, especially domesticated stock in children's zoos, but there is little published information on these programs [32].

Only killed vaccines should be used in zoo animals, unless the vaccine has been specifically tested in the target species [32]. Live vaccines for domesticated animals may be pathogenic in non-domesticated species. For example, carnivores vaccinated with live canine distemper vaccines may become ill. An hAd5-vectored rabies vaccine has been tested in red foxes, raccoons, striped skunks and a variety of non-target wildlife species and laboratory animals, without ill effects [402]; however, hAd5-vectored FMD vaccines would need to be evaluated for safety if they were to be considered in zoo species.

Whether the OIE guidelines for the use of FMD vaccines in domesticated livestock are appropriate for captive non-domesticated species is unknown [32]. There is very little experimental information on the efficacy of FMD vaccines in these animals. One study tested oil adjuvanted, SAT strain vaccine in African buffalo, impala and eland ([403] reviewed in [32]). The vaccine was administered subcutaneously at the cattle dose of 3ml, with a second dose at 21 days, followed by a booster at 6 months. Neutralizing titers to FMDV were lower in these three species than in cattle. It was recommended that two doses of the vaccine be given, 21 days apart, followed by a booster at 4-6 months, with further boosters repeated at 6-month intervals. One FMD vaccine that was produced in the Netherlands in 2002 is supposed to be effective in Asian water buffalo [32]. Vaccine delivery could be a problem in some species, due to concerns about handling the animals. In 1961, the Dutch Central Veterinary Institute developed a special concentrated vaccine formula (aqueous adjuvant) for zoo animals, which could be delivered by projectiles [32]. No side effects such as abscesses or tissue necrosis were seen. NSP ELISAs, which are currently not validated for zoo animals, should be examined for use as DIVA tests.

28. PUBLIC ACCEPTABILITY OF VACCINATION AS A COMPONENT OF FMD ERADICATION

Summary

The general public's attitude toward FMD vaccination may be influenced by opinions on mass culling and animal welfare concerns, as well as by the acceptability of meat and milk from FMD-vaccinated animals in markets. Acceptance of FMD vaccination among the public and producers is expected to vary with the disposition of the animals, i.e., vaccination-to-kill or vaccination-to-live. In some outbreaks, there has been intense criticism by the public and farmers when large numbers of apparently healthy animals were culled. Opposition to mass slaughter sometimes influenced control measures during an outbreak (Uruguay) or future FMD policies (the Netherlands). Attitudes may vary between different types of producers, and stakeholder opinions should be gathered from diverse sources.

FMD is not considered to be a public health threat. FMDV can be zoonotic, but clinical cases in humans are thought to be very rare.

No human health risks are expected from eating meat or milk from animals vaccinated with approved FMD vaccines.

In an outbreak, the possibility must be considered that FMDV might be present in products from vaccinated herds before surveillance is complete. Procedures have been established to inactivate FMDV in various animal products, and the U.K. has developed a marketing plan for meat and milk use after emergency vaccination.

Consumer surveys on eating meat and milk from animals vaccinated for FMD have been equivocal. In the U.K., people surveyed during the 2001 outbreak expressed concerns; however, a survey taken after the outbreak found that people were willing to eat meat or milk from these animals. In general, there are increasing concerns among consumers about food safety and purity, and understanding of the real risks in specific situations may be weak. Measures have been recommended to help minimize the rejection of food from animals vaccinated during an emergency.

Attitudes toward FMD vaccination among the general public may be influenced by opinions on mass culling and animal welfare concerns, as well as by the acceptability of meat and milk from FMD-vaccinated animals in markets. There has been intense public criticism when large numbers of apparently healthy animals were culled during some outbreaks, including the 2001 epizootics in the U.K. and the Netherlands [1-4]. Opposition by farmers was one factor in the decision to implement mass vaccination-to-live rather than continue stamping out during the 2001 epizootic in Uruguay [129]. Concerns about the ethics of killing large numbers of healthy animals were also reported during the 2001 outbreak in Taipei, China [365].

The acceptability of FMD vaccination among the public and producers is expected to vary with the disposition of the animals, i.e., vaccination-to-kill or vaccination-to-live. Vaccination to kill was widely criticized by the public and some farmers in the Netherlands in 2001 [3;4], although trade and agriculture organizations initially supported the choice [4]. In the 2001 U.K. epidemic, farmers stated a preference for culling and earlier compensation, if animals would be killed whether or not they were vaccinated [11]. Concern that milk and meat could not be sold was another factor in rejecting vaccination-to-kill during this epizootic [11]. There is a possibility that attitudes may vary between different types of producers, and stakeholder opinions should be gathered from diverse sources. Large exporters who have safety nets for immediate losses and are more concerned about maintaining the market for their products might be opposed to vaccination, but small-scale operators may be less able to deal with immediate losses of animals [400].

28.1 Foot and Mouth Disease as a Zoonosis

FMD is not considered to be a public health threat [404]. FMDV can be zoonotic, but infections in humans are thought to be very rare [405]. Exposure to extremely large amounts of virus or a predisposing condition may be necessary for infection with this virus [406]. Between 1921 and 1969, more than 40 laboratory-confirmed cases of FMD in humans were published [405]. In three laboratories, 15% to 54% of workers were seropositive, but no clinical cases were reported [405]. One FMD laboratory reported only 2 cases in more than 50 years, and a large FMD vaccine manufacturer documented 3 human cases among its workers [405]. There are no reports of FMDV infections among children who may have been exposed in contaminated smallpox vaccines [405].

Infected humans may be subclinically infected or develop vesicular lesions and influenza-like symptoms [405;406]. If clinical signs are present, the disease is generally mild, short-lived and self-limiting. Broken skin is a recognized route of entry for the virus, with the initial lesions developing at the inoculation site [406]. In 1834, three veterinarians apparently infected themselves by deliberately drinking raw

(unpasteurized) milk from infected cattle for three days [405;407]. No cases of FMD have been reported after eating meat from infected animals [406]. Person-to-person transmission has never been reported [407]; however, vesicles from affected people do contain virus [405]. Reports of FMD in humans have become even more rare since vaccination reduced the incidence of this disease [405].

28.2 The Use of Meat and Milk from Vaccinated and/or Potentially Infected Animals

Vaccines are used regularly in livestock without adverse effects on human health. The U.K. Food Standards Agency has stated that there is no risk to human health from eating animal products after the animal was vaccinated with an approved FMD vaccine [310]. Meat and milk from animals vaccinated for FMD has been consumed routinely in some regions for decades [1]. For 50 years, deboned meat from vaccinated cattle in South America has also been for sale in the U.K. [11].

In an outbreak, the possibility must be considered that FMDV might be present in products from vaccinated herds before surveillance is complete. Procedures have been established to inactivate FMDV in various animal products [70].

28.3 Procedures to Inactivate FMDV in Animal Products

The OIE has published methods to inactivate FMDV in various animal products [70]. These methods reduce the risk to an acceptable level for international trade, although they cannot guarantee zero risk [90;126]. There is no evidence that products inactivated by these methods have been involved in introducing FMDV to any country [126].

FMDV in meat can be inactivated [70] by:

- Canning: The internal core temperature must reach 70°C or greater for a minimum of 30 minutes. Equivalent treatments that have been shown to inactivate FMDV are also allowed.
- Thorough cooking: Deboned and defatted meat should be heated to an internal temperature of 70°C or higher, for 30 minutes or longer.
- Drying after salting: Once rigor mortis is complete, the meat is deboned, salted and dried to a water/protein ratio that is no higher than 2.25:1.

FMDV in milk and cream for human consumption can be inactivated [70] by:

- Ultra-high temperature (UHT) sterilization: Heating to a temperature of at least 132°C for a minimum of one second.
- High temperature - short time pasteurization (HTST): Heating to a minimum temperature of 72°C for at least 15 seconds. This procedure can be used only if the pH of the milk is less than 7.0; however, most milk will satisfy this condition.
- HTST applied twice, if the milk has a pH of 7.0 or higher. It is uncommon for milk to have a pH this high.

More rigorous heating procedures (e.g., HTST applied twice, regardless of milk pH) are used if milk products are to be fed to animals [70], as the risk of infection is higher.

FMDV in casings of ruminants and pigs can be inactivated by salting for at least 30 days with dry salt (NaCl), saturated brine (Aw < 0.80), or a phosphate salt/sodium chloride mixture [70]. The casings should be maintained at a temperature of approximately 20°C during this step.

Procedures have also been established to inactivate FMDV in a wide variety of other animal products such as hides and wool [70].

The limited evidence available, at present, suggests that the risk of importing FMDV in products from vaccinated animals in FMDV-endemic areas is low [126].However, the surveillance and diagnostic

capabilities of the exporting country, and factors such as the probability of infection (e.g., the probability of feeding swill to pigs) and the adequacy of the vaccination program must also be considered [126].

28.4 Procedures for Marketing Animal Products after Emergency Vaccination

In the UK, plans have been established for meat and milk use after emergency vaccination [178]:

- Phase 1 – This stage encompasses the time from vaccination to 30 days after its completion. No movement of vaccinated animals is allowed, except to immediate slaughter after clinical inspection. Fresh milk must be heat-treated as described above. Meat is cross-stamped, transported in sealed containers, and heat treated or naturally fermented and matured, then allowed to enter markets. Once meat has been approved to enter markets, consumers do not see the cross-stamping.
- Phase 2 – During this stage, animals have been vaccinated, but NSP surveillance is not yet complete. Fresh milk is pasteurized. No movement is allowed except to immediate slaughter. Animals sent to slaughter are inspected for clinical signs and tested for antibodies to NSPs. They must not contact other animals during transport or in the slaughter facility. Carcasses must be inspected within 24 hrs after slaughter, and no FMD lesions can be found. Fresh meat from vaccinated pigs is heat treated before marketing. Fresh meat (except offal) from vaccinated ruminants is deboned and matured for intra-Community trade.
- Phase 3 – In this stage, NSP surveillance has been completed but FMD free status has not been yet regained. Slaughter conditions are similar to phase 2, but live animals can be moved between premises by permit. Fresh milk is pasteurized. Meat may be treated, but it can also be sold untreated in domestic markets.

28.4.1 Consumer Concerns about Eating Animal Products from FMD-Vaccinated Animals

Unless consumers can be persuaded that products from vaccinated animals are safe, there may be market shocks from consumer fear even if the disease itself is controlled by vaccination [400]. During the 2001 FMD epizootic in the UK, there were fears that meat and milk from vaccinated animals would need to be labeled and marketed separately [11;408]. Some supermarket chains claimed they would not sell milk from vaccinated cattle [11].

Consumer surveys have been equivocal about the extent of the concern during this epizootic. In surveys during the outbreak, most people had some fears about eating meat from vaccinated animals, and needed reassurance that it was safe [408]. Some consumers, especially mothers with young children, continued to have doubts about the long-term safety of products from these animals. Most people surveyed stated that they would choose nonvaccinated products if the product was labeled, even if they thought vaccination was safe. However, these surveys also suggested that most people felt an alternative to mass culling, such as vaccination, was needed for FMD. In 2003, another study in the U.K. using consumer focus groups concluded that there was no evidence they would have been unwilling to buy or eat products from vaccinated animals ([409] reviewed in[408]). It is, however, possible that people are more cautious during an event than they would expect to be. The latter study also found that the general public seems to relate animal vaccination to human vaccination, and has difficulty understanding the concept of vaccination-to-kill. In addition, it concluded that explaining complex scientific topics to the public during a crisis is impractical, and public education should be addressed before an outbreak. This study suggests that concerns may be heightened during emergencies when there is coverage of the outbreak in the media, and that there is little or no consideration given to eating products from vaccinated animals at other times [408].

In general, there are increasing concerns among consumers about food safety and purity, and the understanding of the real risks in specific situations may be weak [408]. In 2005, the E.U. Directorate-General for Health and Consumer Protection and the European Food Safety Authority (EFSA) commissioned a survey, conducted in all E.U. countries, on the public perception of risk and particularly on food safety ([410] reviewed in [408]). This study found that people were most concerned about factors

such as pesticide residues, new viruses, bacterial contamination and unhygienic conditions outside the home. There were also concerns about animal welfare, genetically modified organisms, environmental pollutants, food additives and other issues. The report did not specifically address vaccination, but it suggests that consumers have a wide variety of concerns about food, with the most concern directed toward issues that are not under the person's control.

Measures that could be taken to minimize the rejection of food from animals vaccinated during an emergency [408]:

- Develop a vaccination policy before an outbreak, and determine the conditions under which it would be used
- Discuss the vaccination policy with all stakeholders. Remind stakeholders that vaccines are used routinely in livestock and poultry for endemic diseases.
- Obtain the support of the public for vaccination and other control policies
- License vaccines before they will be needed. If a conditional license must be given to an emergency vaccine, consider its effect on consumer concerns. Provide safety information to all stakeholders about the use of such vaccines.
- Do not separately label products from animals vaccinated for FMD
- Give unequivocal and authoritative assurance that vaccinated products are safe to eat. This should include statements from national and international independent bodies that consumers respect.
- Begin communication about FMD vaccines before an outbreak and continue to communicate during the outbreak.

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30. ACKNOWLEDGEMENTS

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Glossary

Adjuvant

A substance added to vaccines to enhance the capacity to stimulate the production of antibodies or cell-mediated immune responses.

Animal and Plant Health Inspection Service

Agency within USDA responsible for protecting livestock and plant health.

Animal Husbandry

Basic animal care that is needed to produce a healthy animal.

Animal Product

Blood or any of its components, bones, bristles, feathers, flesh, offal, skins, and any by-product containing any of those components that originated from an animal or bird.

Animals

Livestock, poultry, and all other members of the animal kingdom, including birds whether domesticated or wild, but not including man. (9 CFR 53)

Antigenic Cartography

A computational technique that can be used to visualize and quantify data from binding assays, such as the hemagglutination inhibition (HI) test.

Auction Market

A facility located near livestock concentrated areas that serves as a point to buy and sell livestock. Also referred to as sale barns and livestock markets.

Biosecurity

A series of management practices designed to prevent the introduction of disease agents onto an animal production facility.

Alternate definition: A series of management practices designed to prevent the introduction of disease agents onto or prevents the spread from an animal production facility. (Feedlot, Dairy, Swine manuals)

Bivalent Vaccine

A vaccine containing both H5 and H7 antigens, thus conferring immunity to both subtypes.

Boar

An intact male pig.

Bovine

Common domestic cattle and other members of the Family Bovidae.

Buffer Zone

Zone that immediately surrounds an Infected Zone or a Contact Premises.

Bull

An intact male bovine.

Calf

General term for cattle that have not been weaned from their mother.

Cattle

General term for bovids raised for meat or milk. This term encompasses all ages and sexes of animals.

Cleaning and Disinfection (C&D)

Practices involving a combination of physical and chemical processes that kill or remove pathogenic microorganisms – a combination that is vital for the eradication of disease.

Cold Chain

The system used to ensure that vaccines stay within an appropriate temperature range from manufacturer to the point of administration.

Colostrum

The first milk produced by a cow that has just calved. It is rich in nutrients and proteins that the newborn calf needs to establish immunity against disease pathogens. This milk is non-saleable for human consumption.

Contact Premises

Premises with susceptible animals that may have been exposed to the Foreign Animal Disease (FAD) agent, either directly or indirectly, including but not limited to exposure to animals, animal products, fomites, or people from Infected Premises.

Alternate definition: A premises that has been determined to be related by sound epidemiological evidence to a known infected premises, also referred to as an exposed premises. (In: Mass Depopulation and Euthanasia)

Containment Vaccination Zone

Emergency Vaccination Zone within the Control Area. This may be a secondary zone designation.

Control Area

Consists of an Infected Zone and a Buffer Zone.

Alternate definition: Consists of an Infected Zone and Buffer Zone. Has individual premises quarantine for Infected Premises, Suspect Premises, and Contact Premises and movement restrictions for At-Risk Premises and Monitored Premises. (In: Surveillance, Epidemiology, and Tracing)

Cow

A female bovine that has given birth.

Cull

To voluntarily remove from the herd and sell to a slaughter facility. Sometimes referred to as “market” cattle.

Dam

The female parent of a calf.

Alternate definition: The female parent of a calf, foal, or other offspring.

Decontamination

The process of making a person, object, or environment free of microorganisms, radioactivity, or other contaminants.

Depopulation

A method by which large numbers of animals must be destroyed quickly and efficiently with as much consideration given to the welfare of the animals as practicable. Usually reserved for emergency disease situations for containment of disease.

Differentiating Infected from Vaccinated Animals (DIVA)

A type of vaccine that is marketed with a companion diagnostic kit to distinguish animals vaccinated against a disease from those infected with the natural pathogen.

(Alternate – from L. Elsken, January2012)

DNA Vaccine

Vaccine produced by engineering genes for protective antigens into bacterial plasmids, purifying the plasmid DNA from that of the bacterial host, and administering it to the animal.

Efficacy

Specific ability or capacity of the biological product to effect the result for which it is offered when used under the conditions recommended by the manufacturer.

Elimination

Reduction of an infectious disease's prevalence within a population to zero.

Endemic

Present in a population or geographical area at all times.

Epidemic

An (often suddenly) increased number of cases over a broad geographic area.

Epidemiology

The study of disease in populations and of factors that determine its occurrence.

Epitope

A structural component of an antigen which induces an immune response, and to which antibodies and T cell receptors bind.

Equine

Member of the horse family.

Eradication

Reduction of an infectious disease's prevalence in the global host population to zero.

Euthanasia

Deliberate ending of an animal's life in a manner that causes minimal pain and distress.

Exotic

Not native or indigenous to a country.

Exposed Premises

Premises that have been determined to be related by sound epidemiological evidence to a known Infected Premises, also referred to as Contact Premises.

Feedlot

An area of land where cattle are fattened for harvest. Cattle are kept in groups/pens and fed custom diets that are designed to increase their rate of gain.

Fomite

An inanimate object or material on which disease-producing agents may be conveyed (e.g. feces, bedding, harness, clothes, vehicle tires).

Foreign Animal Disease

A terrestrial animal disease or pest, or an aquatic animal disease or pest, not known to exist in the United States or its territories. (In: Surveillance, Epidemiology, and Tracing)

Hay

A high fiber crop (grass or legumes) that is mowed, allowed to dry in the field and made into bales (square or round) and moved to the dairy to be fed to cows as roughage.

Alternate definition: A high fiber crop (grass or legumes) that is mowed, allowed to dry in the field and made into bales (square or round) and moved to be fed to livestock as roughage.

Hemagglutinin (HA)

Variable avian influenza virus surface glycoprotein and major target of the immune response. The HA1 component is responsible for binding to the cell. HA2 is responsible for fusion of the viral and endosomal membranes, which releases the virus into the cytosol.

Highly Contagious Foreign Animal Disease (FAD)

A disease that spreads rapidly from animal-to-animal as well as herd-to-herd through direct contact, aerosol, oral, fomites, or vector-borne transmission. Highly contagious FADs may be recognized by above normal morbidity or mortality per unit time, where morbidity could be characterized solely by a decrease in production.

Incidence

The number of new cases of disease in a defined population over a specific time period.

Incubation Period

The period of time between infection and the development of clinical signs.

Infected Premises

Premises where a presumptive positive case or confirmed positive case exists based on laboratory results, compatible clinical signs, case definition, and international standards.

Infected Zone

Zone that immediately surrounds an Infected Premises.

Isolation

The complete separation of animals from those that may be carrying an infectious or contagious disease.

Isolation

The complete separation of animals from those that may be carrying an infectious or contagious disease.

Live Vectored Vaccine

Vaccine produced by identifying a protective antigen or antigens for a particular pathogen and then engineering the genes coding for those antigens into another organism that may safely express the antigen in the target species.

Maximum Incubation Period

The longest period which elapses between the introduction of the pathogen into a susceptible animal and the occurrence of the first clinical signs (or other epidemiological evidence) compatible with the FAD agent.

Alternate definition: The longest period that elapses between the introduction of the FAD agent into a susceptible animal and the occurrence of the first clinical signs compatible with the FAD agent. (In Surveillance, Epidemiology, and Tracing Guideline)

Monitored Premises

Premises objectively demonstrates that it is not an Infected Premises, Contact Premises, or Suspect Premises. Only At-Risk Premises are eligible to become Monitored Premises. Monitored Premises meet a set of defined criteria in seeking to move susceptible animals or products out of the Control Area by permit.

Mortality

Death of an animal; dead animals can be referred to as mortalities.

Mortality Rate

The number of deaths in a defined population during a specific time period.

Movement Controls

Control and/or restrictions of the movement of people, animals, vehicles, and equipment so that biosecurity can be maintained during a disease outbreak.

National Veterinary Stockpile (NVS)

Established by Homeland Security Presidential Directive 9 and operational in 2006. Able to deploy large quantities of veterinary resources anywhere in the continental U.S. within 24 hours.

Needle-Free (Transdermal) Injection

Mode of vaccine delivery that uses a specialized system to drive the vaccine into the skin with a burst of compressed air or gas.

Non-Structural Proteins (NSPs)

Viral proteins that are present during replication in cells, but are not packaged into the virion.

Outbreak

An increased number of cases (above what is expected) from a limited geographic area.

Alternate definition: The occurrence of more cases of disease than expected in a given area, or among a specific group, over a particular time period; many epidemiologists use the terms outbreak and epidemic interchangeably. (In: Surveillance, Epidemiology, and Tracing)

Parenteral Injection

Mode of vaccine delivery using a syringe and needle. May be given in the muscle (intramuscularly) or under the skin (subcutaneously).

Pasteurization

The application of heat to food or liquid items (e.g., milk, cheese) to an elevated temperature for a period of time (e.g, 145°F (63°C) for 30 minutes) to destroy certain microorganisms without radically altering taste or quality. This process is considered a mild disinfection process as it does not kill all microorganisms. (Note temp correction made 8/8/11)

Pharmaceuticals

Typically man-made preparations or products that are used for the treatment or prevention of disease. These are regulated by the U.S. Food and Drug Administration (FDA).

Potency

Relative strength of a biological product as determined by test methods or procedures as established by APHIS in Standard Requirements or in the approved Outline of Production for such product.

Poultry

Chickens, ducks, geese, swans, turkeys, pigeons, doves, pheasants, grouse, partridges, quail, guinea fowl, and pea fowl (9 CFR 53).

Premises

Includes a tract of land, and all of its buildings, as well as a separate farm or facility that is maintained by a single set of services and personnel.

Presumptive Positive Case

An animal that has compatible clinical signs, fits a case definition or international standards consistent with a highly contagious FAD in addition to a positive laboratory result indicative of that disease.

Prevalence

The total number of cases of a disease in a given population at a specific time.

Purity

Quality of a biological product prepared to a final form relatively free of extraneous microorganisms and extraneous material (organic or inorganic) as determined by test methods or procedures established by APHIS in Standard Requirements or in the approved Outline of Production for such product, but free of extraneous microorganisms or material which in the opinion of the Administrator adversely affects the safety, potency, or efficacy of such product.

Quarantine

To place animals in strict isolation to prevent the spread of disease.

Rate of Gain

How many pounds of weight an animal gains in a day.

Reservoir

The environment in which a pathogen lives, grows, and multiplies. Can include humans, animals, and the physical environment. The reservoir is often, but not always, the source of infection.

Reverse Genetics

A method used in avian influenza virus vaccine production, which can generate an influenza virus entirely from cloned cDNAs (DNA synthesized from the viral RNA). Reverse genetics allows a vaccine strain to be produced with the HA and NA of choice, with internal proteins from an avian influenza virus strain that grows well for vaccine production (e.g., the human vaccine strain PR8).

Risk

The probability of becoming infected given that exposure to an infectious agent has occurred.

Risk Factor

An aspect of behavior, an environmental exposure, or a hereditary characteristic that is associated with an increase in the occurrence of a particular disease.

Ruminant

Animals (cattle, sheep, goats, deer and camels) with a four-compartment stomach (rumen, reticulum, omasum, abomasum) that digests forages and grains and turns it into energy. Ruminants chew their cud (regurgitate forages from the rumen) to aid in digestion.

Sale Barns

A location for buying, selling, or trading pigs.

Sensitivity

The proportion of true positives that are detected by a diagnostic test.

Serial

The total quantity of completed product which has been thoroughly mixed in a single container and identified by a serial number, provided that, when all or part of a serial of liquid biological product is packaged as a diluent for all or part of a serial of desiccated product, the resulting combination packages shall be considered a serial of the multiple fraction product.

SOP

Standard Operating Procedures that provide specific details related to various topic areas.

Sow

An adult female pig which has had piglets.

Specificity

The proportion of true negatives that are detected by a diagnostic test.

Stability

The ability of a vaccine to remain potent for a period of time, or its “shelf life”.

Stamping-Out

The killing of the animals which are affected and those suspected of being affected in the herd and, where appropriate, those in other herds which have been exposed to infection by direct animal to animal contact, or by indirect contact of a kind likely to cause the transmission of the causal pathogen.

State

Any of the States, the District of Columbia, the Commonwealth of Puerto Rico, Guam, the Commonwealth of the Northern Mariana Islands, the Virgin Islands of the United States, or any territory or possession of the United States (Animal Health Protection Act 2002).

Subtype

Combination of hemagglutinin and neuraminidase type used to describe avian influenza viruses, e.g., H5N1.

Suppressive Vaccination

Emergency vaccination conducted both within and around infected zones. Suppressive vaccination can take place throughout a country or compartment; however, this strategy may require large quantities of vaccine and sufficient human resources.

Surveillance

An intensive form of data recording that encompasses gathering, documenting, and analyzing data. Information is then disseminated so that action can be taken to evaluate disease status and eradicate or control a disease.

Surveillance Zone

Zone outside and along the border of a Control Area.

Susceptible Animal

Any animal that can be infected with and replicate the disease pathogen of concern.

Suspect Premises

Premises under investigation due to the presence of susceptible animals reported to have clinical signs compatible with the FAD. This is intended to be a short-term premises designation.

Targeted Vaccination

Vaccination of selected animals or populations (e.g., uninfected animals of high value including livestock with valuable or unusual genetic backgrounds, long-lived production animals, zoo animals, or endangered species). Can also be directed at uninfected areas where there is a high density of susceptible animals.

Tracing

Information gathering on recent movements (during a defined time period) of animals, personnel, vehicles, and fomites (both to and from affected farms) to identify potential spread of disease to other livestock premises and to detect a putative source of infection for the affected farm.

Vaccination Withdrawal Time

The period between the administration of the vaccination and the time the vaccinated animal or products from that animal can legally enter the human food chain. That period of time is specified in the vaccine product license.

Vaccination Zone

Emergency Vaccination Zone classified as either a Containment Vaccination Zone (typically inside a Control Area) or a Protection Vaccination Zone (typically outside a Control Area). This may be a secondary zone designation.

Vector

Insects or arachnids capable of transmitting pathogens from an infected animal to another animal, usually through a bite.

Alternate definition: An insect or any living carrier that transports an infectious agent from an infected individual to a susceptible individual or its food or immediate surroundings.

World Organization for Animal Health (OIE)

The intergovernmental organization created by the International Agreement of 25 January 1924, signed by 28 countries. In April 2011, the OIE totaled 178 Member Countries. OIE standards are recognized by the World Trade Organization as reference international sanitary rules. The purpose of the OIE is to guarantee the transparency of animal disease status world-wide.

Zoning

The practice of defining subpopulations of animals on a geographical basis, using natural, artificial, or legal boundaries, for the purpose of disease control (OIE).

Zoonotic Disease/Zoonoses

Diseases that are transmissible from animals to humans under natural conditions.

Acronyms

AGID
Agarose Gel Immuno-Diffusion test
APHIS
Animal and Plant Health Inspection Service
BZ
Buffer Zone
C & D
Cleaning and Disinfection
CA
Control Area
CFSPH
Center for Food Security and Public Health
CP
Contact Premises
CSF
Classical Swine Fever
CTL
Cytotoxic T Lymphocyte
CVB
Center for Veterinary Biologics; a division of APHIS
CVO
Chief Veterinary Officer
CVZ
Containment Vaccination Zone
DEFRA
Department for Environment, Food, and Rural Affairs; division of United Kingdom government
DIVA
Differentiating Infected from Vaccinated Animals
DOI
Duration of Immunity
EITB
Enzyme-linked Immuno-electrotransfer Blot
ELISA
Enzyme-Linked Immunosorbent Assay
EUFMD
European Commission for the Control of Foot-and-Mouth Disease
EUVB
European Union Vaccine Bank
FAD
Foreign Animal Disease
FADDL
Foreign Animal Disease Diagnostic Laboratory
FAD PReP
Foreign Animal Disease Preparedness and Response Plan
FAO
Food and Agriculture Organization
FDA
U.S. Food and Drug Administration
FMDV
Foot-and-mouth Disease Virus
FSA
Food Standards Agency
HA
Hemagglutinin
IFN
Interferon
IM
Intramuscular
IN
Intranasal
IV
Intravenous
IVB
International Vaccine Bank
IZ
Infected Zone
LPBE
Liquid-Phase Blocking ELISA
MP
Monitored Premises
NA
Neuraminidase
NAFMDVB
North American Foot-and-Mouth Disease Vaccine Bank
NAHEMS
National Animal Health Emergency Management System
NIMS
National Incident Management System
NSP
Non-Structural Proteins

NVS
National Veterinary Stockpile
NVSL
National Veterinary Service Laboratories
OIE
Office International des Epizooties', currently referred to as the World Organization for Animal Health
PCR
Polymerase Chain Reaction
PD50
Protective Dose
PEL
Policy, Evaluation, and Licensing; a division of CVB
PIADC
Plum Island Animal Disease Center
PPG
Percentage of protection against generalized foot infection
PReP
Preparedness and Response Plan
PVZ
Protection Vaccination Zone
RT-PCR
Real Time Polymerase Chain Reaction
SN
Serum Neutralization
SOP
Standard Operating Procedures
SP
Suspect Premises
SP
Structural Protein
SZ
Surveillance Zone
TCID50
Median Tissue Culture Infective Dose
USDA
United States Department of Agriculture
VAC
Vaccine Antigen Concentrate
VLP
Virus-Like Particle
VMO
Veterinary Medical Officer
VNT
Virus Neutralization Test
VS
Veterinary Services; a division of APHIS
VZ
Vaccination Zone