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

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
Preventing and responding to foreign animal diseases (FADs), like highly pathogenic avian influenza (HPAI) and foot-and-mouth disease (FMD), are critical measures to safeguard our nation’s animal health, public health, and food supply.

There are significant potential consequences of an FAD outbreak in the United States. For example, the 2001 FMD outbreak in the United Kingdom cost an estimated £8 billion ($13 billion) and reduced the British gross domestic product by 0.2 percent. Studies have projected a likely cost of between $6 billion and $14 billion for a U.S. outbreak contained to California. In addition to the economic impact, the social and psychological impact on both producers and consumers would be severe.

Challenges of Responding to an FAD Event
An FAD outbreak will be challenging to all stakeholders. For example, there will be disruptions to interstate commerce and international trade. Response activities are complex, and significant planning and preparation must be conducted before an outbreak. Outbreaks can become large and widespread. Large, geographically dispersed and diverse teams will need to be assembled rapidly and must react quickly. The response effort must have the capability to be rapidly scaled up, involving many times more resources, personnel, and countermeasures. As such, responding to an FAD—large or small—may be a very complex and difficult effort.

Lessons Learned from Past FAD Outbreaks
Past outbreaks both in the United States and other countries have allowed us to learn important lessons that can be applied to preparedness and response efforts. To achieve successful outcomes in future FAD outbreaks, it is vital to identify, understand, and apply these lessons learned:

- Provide a unified State-Federal-Tribal-industry planning process that respects local knowledge
- Ensure the unified command sets clearly defined and obtainable goals
- Have a unified command that acts with speed and certainty to achieve united goals
- Employ science-based and risk-management approaches that protect public health and animal health, stabilize animal agriculture, the food supply, and the economy
- Ensure guidelines, strategies, and procedures are communicated and understood by responders and stakeholders
• Acknowledge that high expectations for timely and successful outcomes require the:
  – Rapid scale-up of resources and trained personnel for veterinary activities and countermeasures
  – Capability to quickly address competing interests before or during an outbreak
• Execute FAD tracing, which is essential for the efficient and timely control of FAD outbreaks

FAD PReP Mission and Goals
The significant threat and potential consequences of FADs and the challenges and lessons-learned of effective and rapid FAD response have led to the development of the Foreign Animal Disease Preparedness and Response Plan, also known as “FAD PReP.” 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 de-conflict 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 protect public health; and (3) provide science- and risk-based approaches and systems to facilitate continuity of business for non-infected animals and non-contaminated animal products.

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. This strategy is provided and explained in a series of different types of integrated documents, as illustrated and described below.

FAD PReP Suite of Documents and Materials

Strategic Plans—Concept of Operations
- APHIS Framework for Foreign Animal Disease Preparedness and Response: This document provides an overall concept of operations for FAD preparedness and response for APHIS, explaining the framework of existing approaches, systems, and relationships.
- National Center for Animal Health Emergency Management (NCAHEM) Stakeholder Coordination and Collaboration Plan: This plan describes NCAHEM strategy for enhancing stakeholder collaboration and identifies key stakeholders.
- NCAHEM Incident Coordination Group Plan: This document explains how APHIS headquarters will organize in the event of an animal health emergency.

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. These documents offer guidance to all stakeholders on capabilities and critical activities that would be required to respond to an FAD outbreak.

Critical Activity Standard Operating Procedures (SOPs)
- For planners and responders, these SOPs provide details for conducting 23 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 strategic documents or disease-specific response plans.

Continuity of Business Plans (Developed by public-private-academic partnerships)
- Secure Egg Supply (SES) Plan: The SES Plan uses proactive risk assessments, surveillance, biosecurity, and other requirements to facilitate the market continuity and movement of eggs and egg products during an HPAI outbreak.
- Secure Milk Supply (SMS) Plan: Currently under development, the SMS plan will help facilitate market continuity for milk and milk products during an FMD outbreak.

Outbreak Response Tools
- Case definitions, appraisal and compensation guidelines and formulas, and specific surveillance guidance are examples of important outbreak response tools.

State/Tribal Planning
- State and Tribal planning is essential for an effective FAD response. These plans are tailored to the particular requirements and environments of the State or Tribal area, taking into account animal populations, industry, and population needs.

Industry, Academic, and Extension Planning
- Industry, academia, and extension stakeholder planning is critical and essential: emergency management is not just a Federal or State activity.

APHIS Emergency Management
- APHIS directives and Veterinary Services Memorandums provide critical emergency management policy. APHIS Emergency Management documents provide guidance on topics ranging from emergency mobilization, to the steps in investigating a potential FAD, to protecting personnel from highly pathogenic avian influenza.

These documents are available on the FAD PReP collaboration website: https://fadprep.lmi.org. For those who have 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.
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. This Appendix A: Vaccination for Foot-and-Mouth Disease provides information for Vaccination Group Supervisors and other personnel associated with vaccination activities. 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. For more detailed vaccination information, consult the FAD PReP Standard Operating Procedures (SOP): 16. Vaccination and plans developed specifically for the incident.
Several key APHIS documents complement this “Appendix A: Vaccination for Foot-and-Mouth Disease Strategies and Considerations” and provide further details when necessary. This document references the following APHIS documents:

- APHIS Framework for Foreign Animal Disease Preparedness and Response Plan (FAD PReP), Chapter 3, USDA-APHIS

- FAD PReP/NAHEMS Guidelines:
  - Vaccination for Contagious Diseases (2011)

- FMD Response Plan: The Red Book, USDA-APHIS

These documents are available on the FAD PReP collaboration website at: https://fadprep.lmi.org
Username and password can be requested.
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 that 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. Serotypes A and SAT are highly variable, but the Asia-1 viruses tend to remain relatively stable in their antigenic types. 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. 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, 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 by direct contact is probably 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
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. Type O is the most prevalent and widely distributed serotype, followed by Asia 1, while serotypes C and SAT 3 are the least common [6;7]. Within these serotypes, more than 65 strains have been recognized [8]. FMDV can also be grouped into topotypes, units that contain closely related strains of the virus.

The most common serotypes and strains vary with the geographic region [9]. Type O, A, C, SAT-1, SAT-2 and SAT-3 viruses are reported from Africa, serotypes O, A, C and Asia-1 have been found in Asia, and serotypes O, A and C have been detected in South America [9]. Serotype C rarely causes outbreaks and seems to have disappeared from some regions [6;9]. Although it still occurs in parts of Africa, this serotype is uncommonly reported in South America, and might no longer be found in Asia. FMD viruses frequently enter the Middle East from both Asia and Africa [9]. Types 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 [6]. The predominant FMDV topotypes in a region sometimes remain stable for long periods [6;9]. However, viruses can also spread into new areas, and new strains can develop spontaneously. Antigenically novel strains of serotype A emerge and disappear regularly in Asia and South America [6]. SAT strains also seem to be highly variable [6]. In contrast, the Asia-1 viruses tend to remain relatively stable in their antigenic types, despite the occasional emergence of new strains [6].

Strains of FMDV can vary in their species preferences, clinical presentation, transmission characteristics and possibly their tendency to become established in carriers [10-13]. 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 [12]. Some topotypes of high threat, such as the Pan-Asia topotype of serotype O, are relatively well characterized. The Pan-Asia topotype was first identified in India in 1990, has spread into most of Asia, and has been responsible for a number of recent outbreaks in FMD-free countries throughout the world [12]. In addition to causing the 2001 epizootic in the U.K., it 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 [7]. Viruses of this topotype affected a variety of species including cattle, pigs, sheep and goats in some outbreaks, but they displayed more limited host preferences in others [6].

Animals that have been infected by, or immunized against, one FMDV do not necessarily have immunity to other strains. There is no cross-protection between serotypes [13]. Within a serotype, protection between strains varies with their antigenic similarity.

### 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. Ranched cervids including deer and elk are also hosts for the virus. Llamas and alpacas can be infected experimentally, but they do not seem to be very susceptible to natural infections [14;15]. Recent studies suggest that Bactrian camels (*Camelus bactrianus*) can develop FMD, but dromedary camels (*Camelus dromedarius*) have little or no susceptibility to this virus [16-18]. FMDV is not known to infect horses, mules or donkeys.
At least 70 species of wild or zoo animals are variably susceptible to FMD [19;20]. Some of the species that can be affected are African buffalo (Syncerus caffer), American bison (Bison 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 strepsicorinis), impala (Aepyceros melampus), tapir (Tapirus spp.), yaks (Bos grunniens), gaur (Bos gaurus), gayal (Bos frontalis), kouprey (Bos sauvelii), mouflon sheep (Ovis musimon), eland (Taurotragus spp.), babirusa (Babyrousa babyrussa), white-tailed deer (Odocoileus virginianus), sika deer (Cervus nippon) and several other species of deer, antelopes and gazelles [19;20]. 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 ([21] reviewed in [20]). There are no reports of FMD in hippopotamus (Hippopotamus amphibius), and serology in South Africa found no evidence of infection in this species [19;20].

Susceptible non-cloven-hooved animals include hedgehogs (Erinaceus sp.), armadillos, kangaroos, nutrias (Myocastor coypus) and capybaras (Hydrochaeris hydrochaeris) [19;20;22]. 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 the latter species is not considered susceptible to FMD under natural conditions in southern Africa [19]. There is a report of a fatal FMDV infection in one crested porcupine (Hystrix cristata); however, the diagnosis was made solely by histology, based on myocardial necrosis, with no other proof that FMDV was involved ([21;23] reviewed in [20]). FMD was also reported in a grizzly bear (Ursus arctos horribilis) in a South American zoo, but no virus isolation or serological analysis was done ([24] reviewed in [20]). Because there are no other reports of FMD in bears, this diagnosis is suspect [20]. Laboratory animal models include guinea pigs, rats and mice, but these species are not important in transmitting FMDV in the field [19].

Cattle are usually the most important maintenance hosts for FMDV except in Africa, where African buffalo maintain SAT type viruses [13;25]. There is also evidence that some FMDV isolates might circulate in populations of Asian water buffalo [26;27]. Some viral strains may primarily be found in pigs, sheep or goats [13]. The pig-adapted type O Cathay strain (Hong Kong topotype) has not infected large ruminants in outbreaks, and it does not grow in ruminant cells on primary isolation [13]. This strain has been isolated only once from a bovine [28]. 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 [28]. Some other serotype O strains are well adapted to sheep and goats, although they can also affect cattle [29]. However, it is uncertain whether small ruminants can maintain FMDV for long periods if cattle are absent [13;29-31]. 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 [10;13].

With the exception of African buffalo, wildlife hosts do not seem to be able to maintain FMDV for more than a few months if domesticated livestock are not infected [13;19]. 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 [19]. Outbreaks can, however, occur for short periods among wildlife. In 1924-1926, FMDV was speculated to have spread from cattle to wild mule deer (Odocoileus hemionus) in California ([32] reviewed in [20]). 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. In the late 19th century, many impala died of FMD in South Africa, and an outbreak killed half of the population of wild mountain gazelles (Gazella gazella) at a nature reserve in Israel in 1985 [19;20;33]. The potential for feral populations of domesticated animals (e.g., feral swine) or wild relatives of domesticated species to maintain FMDV should also be considered.
3.3 Pathogenesis
FMDV is thought to replicate at a primary site before it disseminates. In cattle infected via aerosols, this location appears to be the nasopharynx [34]. Replication at the primary site is followed by viremia, usually accompanied by a fever, with dissemination to secondary replication sites [7]. In cattle infected by aerosols, viremia coincides with the replication of virus in pneumocytes in the lungs and decreased replication in the nasopharyngeal tissues [34]. Viremia lasts 2-3 days, and ends when circulating antibodies appear ([35] cited in [7]). The secondary replication sites for FMDV are primarily stratified, cornified squamous epithelia [10]. 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 [10;28;29;36]. In some cases, replication can peak as early as 2-3 days after infection [10]. FMDV is usually eliminated from secondary sites of replication within 10-14 days ([37] cited in [7]). 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 [38]. In cattle, clinical signs appear in two to 14 days, depending on the dose of the virus and route of infection [39]. In pigs, the incubation period is usually two days or more, but it can be as short as 18-24 hours, and may be as long as 9 days [28]. 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 [10;29;40]. Vesicles develop in limited locations, usually on the feet, in and around the mouth, and on the udder [14;19;28;29;39;41]. Occasionally, they may be found at other locations 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 may be sloughed. Most adult animals recover within two to three weeks, although secondary bacterial infections may lead to a longer recovery time [14;41]. Among domesticated animals, deaths usually occur mainly in the young, as the result of multifocal myocarditis; vesicles are not always found in these cases [13;14]. In some outbreaks, the mortality in young animals can be very high [14;28;29;39;42]. 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 [41]. High fatality rates have occasionally been reported in some species of wildlife or zoo animals [19;20].

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 [39]. 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 [39]. Cattle typically become febrile and develop lesions on the tongue, dental pad, gums, soft palate, nostrils or muzzle, and sometimes the teat [14;39;41]. 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 [39].

In pigs, the most severe lesions usually occur on the feet [14;28;41]. 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 [28].

In contrast to pigs and cattle, the clinical signs in sheep and goats tend to be mild [14;29;41]. The signs can vary with the topotype. 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. Approximately 25% of infected sheep remain asymptomatic, and 20% have a single lesion [29]. Minimal lesions and fever have been reported in llamas, which rarely become anorexic or demonstrate pain and discomfort [14].

The clinical signs in wildlife resemble those seen in domesticated livestock [19]. 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 wart hogs. 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. Severe illness has been documented in a population of mountain gazelles, as well as in impala, blackbuck, white-tailed-deer, pudu, warthogs, a kangaroo and some other species [19;20]. Young animals of any species can die suddenly of myocarditis [19].

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 [10;13;41]. Animals can shed the virus for up to four days before the onset of clinical signs [39]. FMDV also occurs in vesicle fluid, and large quantities of virus may be shed when the vesicles rupture [10;28;29;36]. There are some species differences in the timing of virus shedding: in sheep, maximal virus excretion occurs 1-2 days before the animals develop clinical signs, while in cattle and pigs, maximal shedding is around the time of vesicle formation [6;11]. FMDV can be transmitted to other animals by direct contact, or by indirect contact via aerosols or contaminated fomites and environments [13;14]. The 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 [28;29;39]. 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 [10;14;19;43].

The amount of aerosolized virus produced varies with the strain of FMDV, and airborne transmission is more important for some topotypes and strains than others [11;12]. 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 topotype of serotype O [12]. In some locations, there seems to be little or no aerosol transmission of pig-adapted O Cathay viruses between herds [6]. Airborne transmission is influenced by climatic conditions, and FMDV also spreads much farther over water than land [44]. 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 [12]. Aerosol transmission over land alone is said to be rarely greater than 10 km [12]; 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 [45]. An airborne plume was reported to spread the virus 60 km during the 1967-68 outbreak in the U.K [11].

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 [46]. Virus stability increases at lower temperatures, and in very cold climates, survival up to six months or more may be possible. 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 [46]. Organic material protects FMDV from drying, and enhances its survival on fomites. Virus survival is also improved under conditions where it is protected from sunlight. FMDV is inactivated at pH below 6.0 or above 9.0 [41]. 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 [6;41;47]. However, acidification does not occur to this extent in the bones and glands, and FMDV may persist in these tissues [6].

Seasonal changes in animal movements and trading patterns can lead to seasonality in FMD outbreaks [48], or to an increased risk of epizootics from an introduced virus at certain times of the year [49].

FMDV may be carried mechanically in the nares of uninfected humans for short periods, but how long this can occur is still uncertain. 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 [50]. “No contact” periods for responders in FMD outbreaks have been based on this study. More recent research found that people did not transmit FMD viruses (serotypes O/UK/35/2001 and O/TAW/97) to pigs or sheep when personal hygiene and biosecurity protocols were followed, and suggest that nasal carriage of the virus might be unimportant in transmission [51;52]. 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 [51]. In the second study, FMDV was not isolated from the nares [52]. 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 serotypes Asia1 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 [53]. 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 [53]. Eight people were tested 2-3 days after exposure, and no PCR-positive nasal samples were found [53]. 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.

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 will 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 [39]. Because cattle have a large respiratory volume, and the infectious dose by inhalation may be as low as 20 TCID50, they are particularly susceptible to infection by aerosols [39]. Airborne FMDV may infect this species either from nearby animals or over longer distances. Calves may 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 [39]. Cattle generate up to log10 5.1 TCID50 of aerosolized virus
per day, and a large herd can produce enough viruses to infect neighboring herds [39]. Peak shedding of up to $10^{6.7}$ TCID$_{50}$ per ml occurs in milk, with as much as $10^{6.2}$ TCID$_{50}$ per ml in semen, $10^{4.9}$ TCID$_{50}$ per ml in urine and $10^{5.0}$ TCID$_{50}$ per ml in feces [39]. 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 [39]. Often, 90% of the herd may eventually be affected [6]. 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 are less likely to become infected by aerosols [29]. 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. Transmission between nonvaccinated sheep seems to occur less readily than between cattle or pigs [29]. In some cases, only a small percentage of the flock becomes seropositive or sheds virus. There are cases where only 25% of the animals were infected before the virus disappeared from the flock [6]. Sheep produce much less aerosolized virus than pigs, and they are unlikely to transmit FMDV by aerosols farther than 100 meters [29]. Transmission can occur subclinically in sheep flocks, or with limited lesions, and there is a significant danger that infected flocks might not be detected [29].

It is still uncertain whether small ruminants can maintain FMDV in the absence of other infected species [13;29;31]. 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 [31]. 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 ([30;54] cited in [31]). 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 ([55;56] cited in [31]). 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 ([30] cited in [31]). 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 [57]. 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 [29].

3.6.3 Pigs

Pigs are usually infected by direct contact with infected animals or heavily contaminated environments, or by ingestion of the virus [28]. The infectious dose might vary with the individual pig, and possibly the strain of FMDV. The oral infectious dose is approximately $10^5$ TCID$_{50}$, and may be lower if the animal has mouth lesions. Pigs are relatively resistant to infection via aerosols, compared to ruminants [11;28]. 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 studies suggest that pigs might not become infected if they are physically separated from infected animals [28]. 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 [6]. Swine herds can produce extensive plumes of aerosolized virus [11;44]. This species sheds large amounts of FMDV in respiratory secretions, and can produce $10^5$ 5.8 TCID$_{50}$ to $10^7$ 7.6 TCID$_{50}$ per pig in 24 hours [28]. 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 [11]. 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 [28]. 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 llamas. 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 very 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. 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 vaccination.

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 [58]. 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 [10]. FMDV persists mainly in the pharyngeal region, especially in the dorsal soft palate, and possibly at other sites [10,25]. It is not certain whether this virus is cell-free, possibly in immune complexes, or if it is cell-associated [10]. 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;10].

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;10;13;43;59]. Persistent infections have also been reported in some water buffalo for up to a year [8]. Sheep and goats seem to become carriers less often, and for a shorter time [43]. 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 [43;60]. The longest reported carriage in goats is four months [1;10;60]. Llamas do not seem to become carriers [25;43]. 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;10;25;43].

The current consensus is that pigs do not become carriers [10;25;43], although two reports have suggested the possibility ([61] cited in [62]; and [63]). In particular, Mezencio et al. reported FMDV RNA in the blood of recovered pigs, and fluctuating virus neutralization activity associated with these episodes [63]. A recent study found that one 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 [62]. 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.

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 [43]. 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 white-tailed deer, fallow deer (Dama dama), sika deer and kudu, and occasionally in red deer (Cervus elaphus) [19;43]. 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 [43]. FMDV has also been detected for up to 5 weeks in several white-tailed deer, and one deer carried the virus for 11 weeks [43]. It could be found for up to 57 days in sable antelope (Hippotragus niger) and for nearly 5 months in kudu [43]. 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 [43]. There is no evidence for carriers among impala, which are commonly affected by outbreaks in southern Africa [43].

4.1 Can Carriers Transmit the Virus to Other Animals?
The epidemiological significance of carriers among domesticated livestock is very controversial [10;43;64]. 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 [10;19;43]. 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 ([65] reviewed in [43]). 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 ([66] reviewed in [43]). In both this report and an earlier one ([67] reviewed in [43]), 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 [68]. 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 [10;19;69]. However, the incidence seems to be low, unless the animals are in close contact. For example, there no evidence that FMD
occurred in domestic animals in Botswana for 8 years, although the virus has been found in 50-70% of wild African buffalo in that country [10;43]. Carrier cattle may also be able to transmit SAT viruses [10;25]. Transmission has been reported between cattle carrying SAT-2 viruses after outbreaks in Zimbabwe in the 1980s [25]. 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 [10;25;43].

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 [10;25;43]. 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 [10]. 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 [43]. 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 [10;25;43;70;71]. Some published experiments treated animals with dexamethasone, stressed them, or co-infected them with bovine herpesvirus-1 (infectious bovine rhinotracheitis virus) or rinderpest [10;25;43]. Corticosteroid treatment appears to actually decrease the amount of virus in probang samples from carriers [25;43]. Furthermore, trauma to the feet or infection with Ascaris spp. was unable to increase the susceptibility of pigs to infection from carriers [43]. Nevertheless, the possibility of transmission from carrier cattle or sheep cannot be definitively excluded [10]. 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 [70].

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 [72;73]. 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 [72;73]. It should be noted that, in South America, continued use of prophylactic vaccination might have mitigated the risks (if any) from carriers [74]. Vaccination was also a component of FMD eradication in the past in Europe, and it has recently been a part of successful eradication programs during outbreaks in Albania, Macedonia and other countries [75]. 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 ([76] 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 [43]. 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 ([77] cited in [31]). In contrast, a survey in Asian Turkey in the early 1990s reported that the prevalence of carriers among cattle and sheep was 15–20% ([78] cited in [10]). In Brazil, more than 50% of cattle were carriers in the early 1960s before vaccination was common [43]. This number was greatly reduced by intensified vaccination campaigns, and very few carriers were found in endemic areas by the mid-1980s [43]. 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 ([54] cited in [43]).

Some experimental studies also suggest that vaccination may decrease the percentage of carriers by reducing exposure to the virus [43;79-85]. 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 [80]. In this experiment, the two highest doses of a potent vaccine completely prevented sheep from becoming carriers. In another study, nine of 12 nonvaccinated lambs that were inoculated directly with FMDV and three of 12 nonvaccinated lambs that were exposed by contact with infected animals became carriers, while only one of 24 vaccinated lambs exposed by contact or inoculation was infected after 28 days [31]. Madhanmohan et al. reported that 26% of 23 vaccinated sheep and all 6 nonvaccinated sheep became carriers when challenged after 3 weeks [84]. 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 [82]. 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 [81].

Carriers also 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 [85]. Similarly, Doel et al. reported that more cattle became carriers if challenged soon after vaccination (e.g., 4 days), compared to cattle that were challenged at later time points [79].

5. DETECTION OF INFECTED ANIMALS

<table>
<thead>
<tr>
<th>Summary</th>
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<tbody>
<tr>
<td>Virological tests used to detect acutely infected animals include ELISAs to detect viral antigens, virus isolation and RT-PCR.</td>
</tr>
<tr>
<td>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.</td>
</tr>
<tr>
<td>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.</td>
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<tr>
<td>Vaccination primarily induces antibodies to SPs, and tests that detect titers to NSPs can recognize infections with field viruses in vaccinated animals. Insufficiently purified vaccines can contain low levels</td>
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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. Currently, NSP tests are not reliable enough in individual animals to be prescribed tests for international trade, but 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 the enzyme-linked immuno-electrotransfer blot (EITB). In cattle, the NSP protein 3ABC seems to be the most reliable marker to distinguish vaccinated animals from those infected by field strains, and most NSP ELISAs are based on this protein. A number of commercial 3ABC ELISA kits, as well as some “in house” tests, are available. The specificity and sensitivity of some ELISAs have been published. The sensitivity can differ between vaccinated and nonvaccinated animals, as well as within different categories of infected animals (e.g., subclinically infected compared to symptomatic animals, or carriers compared to transiently infected animals). Reliable ELISAs based on the detection of 3AB proteins have also been developed.

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. Some authors feel that this test is very difficult to use and interpret. 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. The 3ABC ELISA and EITB have been validated mainly in cattle. Their validation in other species is incomplete or absent.

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.

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 [13]. 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 [13;86].

Carrier animals can be definitively identified by recovering FMDV from esophageal-pharyngeal fluids, using virus isolation [13;25]. 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 [25]. Recovery is also influenced by the handling of the sample, and the skill of the person recovering the virus [25]. 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 non-specific inhibitors. It is still uncertain whether recovering only RNA and not live virus should be interpreted as evidence for persistent infections [36]. 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 [25;62]. 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 [25].

### 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 [87]. 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, 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. The 3D protein, which is also called Virus Infection Associated Antigen (VIAA), is a viral RNA-dependent RNA polymerase [25;87]. This protein is often incorporated into the capsid and cannot be purified from
conventional inactivated vaccines [87;88]. Protein 3B (Vpg) is also necessary for the replication of the viral RNA [87]. The protease 3C is required for capsid assembly, and is used in the generation of empty capsids in adenovirus-vectored FMD vaccines [87;89]. Lpro and 2A are also proteases involved in cleaving the viral polyprotein, but they are not necessary for capsid assembly [7;87;89]. In addition, Lpro and 3C cleave specific host proteins [7;87]. Proteins 2B, 2C, and 3A have been implicated in membrane rearrangements that produce the cytoplasmic vesicles where FMDV replicates [90]. Protein 3A is formed from the precursor protein 3AB, which also has a role in virus replication [87].

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 [87], and titers to these proteins seem to be correlated with the extent of virus replication [36]. 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 [25;85;87;91].

Seroconversion to structural proteins (SPs) occurs earlier than to NSPs, and the titers are higher [73]. In cattle, antibodies to SPs have been detected as soon as 3–4 days after infection [87], while antibodies to the NSP proteins 3A, 3B, 3D and 3ABC have been found as early as 7–10 days [87;92;93]. In one study, all cattle had titers to structural proteins on day 8, and developed antibodies to NSPs beginning on days 8-10 [93]. 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. In pigs, Chen et al. first detected antibodies to NSPs 6 to 8 days after infection [94]. Other reports also suggest that these antibodies can be recognized in pigs within the first 1–2 weeks [87]. Titers to structural proteins may persist for the life of the animal, but antibodies to NSPs decline and become undetectable sooner [87]. The immune response to 3ABC appears to persist longer than antibodies to other NSPs, with detectable titers reported for 1-2 years in three studies [87]. 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 may be found in some cattle for up to a year [25;73;87].

Vaccination primarily induces antibodies to structural proteins [87]. 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 [25]. 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 [73;85;87;95-97].

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 [13]. 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 [73].

5.2.4 Serological Tests that Detect Antibodies to Structural Proteins

Serological tests based on structural proteins are serotype-specific [13;38]. 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, recommended serological SP assays include the virus neutralization test (VNT), the solid-phase competition ELISA (SPCE) and the liquid phase blocking ELISA (LPBE) [13]. VNT, which uses live virus and requires cell culture facilities, takes 2–3 days to complete. ELISAs are faster, 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 [13]. Because NSPs are conserved across serotypes and strains, a single assay can recognize infections with all FMD viruses [13]. 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 [87]. 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 [25;73;85;87;91;95-97].

In the OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals, the recommended NSP assays are ELISAs and the enzyme-linked immuno-electrotransfer blot (EITB) [13]. Studies in experimentally infected cattle have reported that 3ABC is the most reliable marker to distinguish vaccinated animals from those infected by field strains [92;98], and most NSP ELISAs are based on this protein. A number of commercial 3ABC ELISA kits, as well as some “in house” tests, are available.

The specificity and sensitivity of some ELISAs have been published. 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 [96;97;99]. In one study, the estimated specificity for six 3ABC ELISAs in vaccinated and nonvaccinated cattle ranged from 97% to 98.5%, and improved to 98-100% when samples were retested using the same assay [99]. 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 NCPantaflota from PANAFTOSA, 3ABC trapping-ELISA from IZS-Brescia and Ceditest® FMDV-NS (Cedi Diagnostics, now 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%) [99]. Another study reported similar results. Engel et al. reported specificities of 96% to 99% for six 3ABC ELISAs in vaccinated and non-vaccinated cattle [97]. 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 [96]. In the latter study, 3 ELISAs were less sensitive when more time had elapsed since infection, but the Ceditest FMDV-NS and an in-house test (Istituto Zooprofilattico Sperimentale, Brescia) were unaffected [96]. Only one of the studies examined 3ABC ELISAs for use with sheep and pigs, and only limited numbers of serum samples were available [99]. In sheep, the
specificity of four ELISAs was reported to be 98% to 100% [99]. 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 [99]. Reliable ELISAs based on the detection of 3AB proteins have also been developed [13]. In Taiwan, a blocking 3AB ELISA was used to detect infected pigs during a vaccination campaign in 1997 [91]. 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 [13;73;87;89;94]. Retesting positive samples, using combinations of ELISA tests to increase specificity, has also been described [74;99].

The EITB uses immunoblotting (western blotting) to detect antibodies to 3A, 3B, 2C, 3D and 3ABC [13]. 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 [13]. If neither case applies, then the interpretation is indeterminate. Some authors feel that the EITB test is very difficult to use and interpret [7]. 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 [73]. 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 [73].

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 are not reliable enough in individual animals to be prescribed tests for international trade, they are valuable as herd tests, and can be used as part of the procedure to regain FMD free status [13;25;38;73;87;100]. 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) [101].

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 [38]. 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 [74]. 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 [74].

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 [73;87]. 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 [101]. 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 [38;74].
The OIE-recommended investigation of herds with seropositive animals includes the use of clinical signs, epidemiological studies and supplementary laboratory tests [38]. 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 [13;38]. The OIE also recommends that virological tests be employed whenever possible. Virological testing (probang samples) of carriers can be difficult to achieve with good sensitivity [74]. 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 [38]. 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. Sentinel animals of the same species (young, nonvaccinated animals or animals with no maternal antibodies) can be tested by serological assays. 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. 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 [38]. 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 [38]. 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 [73]. In the E.U., the European Directive on FMD Control mandates that either all vaccinated animals are sampled, or the sample must detect a within-herd prevalence of 5% at 95% confidence [74]. Combinations of ELISA NSP tests have been described that would allow a 5% prevalence of carriers to be detected at 95% confidence [74]. Some combinations required that the herd contain at least 30 cattle, while others necessitated that it have more than 50 cattle [74]. 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 ([102] cited in [89]; [74;103]). 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 [74]. 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 [74]. 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 [74]. 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% [74]. A 2008 modeling study from Arnold et al., however, suggests that the small herd problem may not exist [64]. 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.
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 [74;101]. 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 [64;104]. 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 [104]. 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 [64]. 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 [64]. 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. Paton et al. 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 [74]. This approach would need to be justifiable to the OIE, in order to substantiate freedom from infection.

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 [10;25;43]. If viruses continue to circulate in vaccinated populations, the evolution of new variants might favor the development of vaccine resistance [105]. Whether carriers can promote the evolution of FMDV strains is still unclear [7].

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 [100]. Different tests have different levels of validation. The 3ABC ELISA and EITB have been validated mainly in cattle [25;38;87]. Their validation in other species is incomplete or absent.

Some 3ABC tests tested in sheep have been able to detect infections among both vaccinated and nonvaccinated animals [31;55;80;85;106]. 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 [85]. Brocchi et al. reported sensitivities of 100% for four NSP ELISAs in nonvaccinated sheep, but only 33% to 67% in vaccinated sheep [99]. Test specificities did not differ between vaccinated and nonvaccinated sheep, and ranged from 98% to 100%.

A few studies have also 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 [62]. No single NSP test detected all infected pigs, but by combining tests, sensitivity and specificity could be increased [62]. Another study, which compared the same three tests, concluded that the 3ABC NSP ELISA from CEDI Diagnostics had the best profile, based on the highest sensitivity and specificity, and the least reactivity with residual NSPs in vaccinated pigs [94]. Brocchi et al. reported that five 3ABC NSP ELISAs had sensitivities of 100% in nonvaccinated pigs, but only 44% to 69% in vaccinated pigs [99]. Test specificities did not differ between vaccinated and nonvaccinated pigs, and ranged from 97% to 100%.
5.2.8 Serological Assays in Development

A serological assay that uses liquid array technology to measure the relative responses to multiple FMDV protein signatures (3A, 3B, 3D and 3ABC) is in development [7;107-109]. The 3ABC signature in this multiplexed DIVA assay is comparable in its performance to a widely used commercial assay. The test also provides information about the relative diagnostic sensitivity of each protein signature.

Serological tests to detect the NSP protein 3D can be used in nonvaccinated animals, or in animals immunized with recombinant vaccines that do not contain this protein, such as Ad5-vectored FMD vaccines (below) in development [7]. A 3D liquid phase blocking ELISA has been developed for cattle and pigs [7]. Antibodies to 3D can also be detected with a traditional agarose gel immuno-diffusion (AGID) test, which has a long turnaround time [7]. 3D tests are not useful in animals vaccinated with conventional inactivated vaccines, which always contain this protein even when they are purified [87;88].

FMD-specific IgA, which occurs in the saliva of recovered or vaccinated cattle, might be useful in the detection of carriers [25]. 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 [25]. However, the levels of specific IgA are not elevated in some individual carriers and this system still requires development.

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. They may also have some advantages, which might include a longer duration of immunity, less interference from maternal antibodies and a better shelf-life.

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 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. Manufacturers vary in their production methods and quality control tests.

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.

If a new vaccine must be prepared from an outbreak strain, the field virus must first be adapted to culture. If the adaptation is successful, the lead time for vaccine preparation is 1 to 6 months.

Human adenovirus 5–vectored empty capsid FMD vaccines, and companion DIVA tests, are being evaluated for manufacturing and licensing in the U.S. If approved, these vaccines could also be stored in the NAFMDVB. Other approaches have not yet produced any vaccines that are close to licensing.

### 6.1 Types of FMD Vaccines

Conventional live attenuated vaccines are unacceptable for FMD [13]. When attempts were made to produce such vaccines in nonsusceptible hosts, the attenuated viruses tended to revert and become virulent [89;110]. 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 [13].
Nearly all currently licensed FMD vaccines are inactivated (killed) vaccines containing chemically inactivated virus [87]. 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. 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

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;7;36;59]. Oil adjuvanted FMD vaccines are at least as effective as aluminum hydroxide adjuvanted vaccines, with some studies suggesting that the duration of immunity is longer [7;59;111] and that there may be less interference from maternal antibodies [13]. Vaccines with oil adjuvants are simpler to manufacture and have a better shelf-life [1;36]. Improved antibody responses and potency have been reported for double oil emulsion compared to water-in-oil single emulsion vaccines [7;112-114].

Experimental vaccines including adenovirus-vector subunit vaccines (see “experimental vaccines,” below) are also in development.

**6.2 Production of Conventional Inactivated FMD Vaccines**

The production of large quantities of FMDV requires high containment BSL-3 (containment Group 4) facilities [7;13]. It is illegal to possess live FMDV on the U.S. mainland, and conventional inactivated vaccines cannot be manufactured in the U.S. [95]. 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 conventional inactivated FMD vaccines that have been manufactured in foreign countries [7]. 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 [7]. 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. FMD vaccines that can be made without live virus could be produced and licensed in the U.S., and efforts are underway to develop such vaccines [7].

Most modern inactivated FMD vaccines are produced in BHK-21 suspension cell cultures [87]. 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 [87]. Formaldehyde was replaced by aziridines (e.g., ethyleneimine, usually in the form of binary ethyleneimine) in the 1970s [7;13;87]. Time and temperature conditions for inactivation must be validated for the conditions and equipment [13]. The virus is usually inactivated twice for safety. The inactivated antigens can be concentrated by polyethylene glycol precipitation, ultrafiltration, or cycles of adsorption and elution using polyethylene oxide [13;87]. 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.

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, [7;59;88] but vaccine bank antigens frozen at –70°C or lower can be stored for at least 5 years [88] and in some cases, for many years [13]. FMD vaccines are formulated by dilution in a suitable buffer, with the addition of adjuvants and other vaccine components [13]. 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) [13]. These vaccines are considered to be temperature labile, and should not be frozen or stored above a target temperature of 4°C [13]. However, preliminary evidence presented at the 2010 European Commission for the Control of FMD meeting suggests that freezing might not adversely affect vaccine potency, and might extend the shelf-life [115]. 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 [115]. It might also 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].

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 [7;79;116]. Serotype O is genetically diverse, but antigenically restricted, and two vaccine strains can immunize against all currently circulating strains [117;118]. Serotype A and SAT viruses are genetically and antigenically diverse, and multiple vaccine strains are needed for immunization [117;118]. SAT-1, SAT-2, and SAT-3 viruses are also less stable than other serotypes [7]. To ensure that vaccines containing the SAT serotypes are potent and remain so during storage, extra quality assurance steps must be taken [7]. Serotype Asia 1 is antigenically homogeneous, and only one strain of Asia 1 and Serotype C are recommended for immunization and vaccine banking [117;118].

6.3. 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 [88;119]. 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 Ad5-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 [59;119]. As of 2010, the NAFMDVB has never been activated. The first activation of the EUVB was for an outbreak in the Balkans in 1996 [59]. The EUVB also supplied vaccines to Japan (which did not use the vaccine) and the Republic of Korea in 2000, and to Turkey in 2000 and 2006 [59;88;119]. The International Vaccine Bank (IVB) (disbanded in 2003) was located in the UK. It had only one large-scale activation, for the 2001 epizootic in the U.K., and the vaccine was not used [59]. 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 [120]. It has also supplied vaccine to Uruguay [119].
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 [59;117]. The shelf life of FMDV antigen concentrates under the storage conditions in vaccine banks is expected to be well over 15 years [13]. The integrity of the antigens must be maintained while they are frozen, stored, thawed and diluted [88]. During storage, some virus particles rupture or aggregate [88]. 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 [88]. After 14 years, as much as 40% of the antigen mass may be lost in some cases [88]. The stability of FMDV antigens seems to be strain-dependent [88]. Manufacturers vary in their production methods and quality control tests. Vaccine banks routinely monitor the storage temperature and periodically inspect bottles, and they may weigh antigen deposits annually to ensure that they have not become lyophilized [13]. Some FMD vaccine banks also do physico-chemical tests such as sucrose density gradient analyses to monitor virus integrity/antigen stability, and some perform periodic in-vivo testing of stored antigens [13].

In an outbreak, FMD vaccine banks can rapidly formulate stored antigens into complete vaccines. These vaccines can be tailored to the epidemiology of the outbreak [59;88]. 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 [88]. 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 [118].

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 [59]. 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;59]. 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 [59]. 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 [118]. 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 standardization and certification.

6.3.1 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 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 [7]. 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 (see section 6.6 for details on the requirements for full and conditional licensure) [7].

6.4 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 [59]. 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 for the Netherlands, which was able to vaccinate immediately with a conventional vaccine after the E.U. approved the use of vaccination [1].

6.5 New Vaccines from Field Viruses
If a new vaccine must be prepared from an outbreak strain, the field virus must first be adapted to culture [7]. Some FMD viruses may be difficult to adapt, or the process may result in changes in antigens [7;13]. If the adaptation 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;118]. If the new vaccine is needed quickly, an emergency release may allow it to be used without necessarily finishing all aspects of control testing.

6.6 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 [121]. 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 [121].

For a vaccine to be given a full product license, the manufacturer must conduct extensive efficacy, purity and safety testing [119;121;122]. 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 [121]. 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 as well as 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 [121]. 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 [59;121].

6.7 Experimental Vaccines
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. [7].

6.7.1 Ad5-Vectored Empty Capsid FMD Vaccines
Human adenovirus 5 (Ad5)-vectored empty capsid FMD vaccines are being evaluated for manufacturing and licensing in the U.S. [7]. These vaccines are being developed in collaboration with DHS and an industry partner, GenVec. The vaccine construct contains the complete capsid coding sequences and the FMDV 3C protease, which is necessary for capsid assembly, in a live replication-defective human
adenovirus vector [89;123-126]. It does not include the genes for NSPs other than 3C. The result is a capsid without infectious FMDV nucleic acids. Because no live FMDV is involved, these vaccines can be manufactured in the U.S. Experimental batches are being made on the mainland, and tested in cattle at the PIADC as part of the licensing process. If these types of vaccines are successful, it would be possible to produce vaccines from field strains without adapting viruses to cell culture [7]. Ad5-vectored FMD vaccines are made as ready-to-use products [7]. They are expected to have a shelf life, when stored frozen, of at least 3 years. Full evaluation of each construct for U.S regulatory approval usually takes 3-5 years. Because most NSPs are absent, Ad5-vectored FMD vaccines can be used with a variety of DIVA tests including 3D assays and the 3ABC ELISA [7;89;208]. Although the 3C protein is produced by this vaccine, seroconversion does not seem to occur in the 3ABC ELISA [208]. Rare false positives have been identified among animals; however, these animals have always been seropositive before vaccination [208]. A 3D ELISA companion test is also in development [7].

The adenovirus vector in these vaccines has both advantages and disadvantages. One advantage is that the viral tropism includes the respiratory tract, which is a site of FMDV replication in infected animals [127]. This might result in the induction of mucosal immunity and cell-mediated immune responses, in addition to neutralizing antibodies. Adenoviruses also have an adjuvant effect when used in vaccines [128]. These viruses are potent inducers of interferon, and can stimulate a variety of inflammatory responses. However, the immune response to the vector could limit the vaccine’s efficacy if there is pre-existing immunity to similar vaccines, or if multiple doses must be given [128]. An experiment in pigs indicated that this might be a concern, when the vaccine was given 2 weeks after injecting the vector alone [124]. In cattle, titers to the vector tend to peak 2 weeks after vaccination, and a second dose of Ad5-vectored FMD vaccine, given after the titers had declined, was able to boost the immune response to FMDV [7].

Published studies of efficacy for Ad5-vectored FMD vaccines have been promising. In one study, pigs vaccinated with a serotype A24 Cruzeiro construct and challenged by inoculation with homologous virus 7, 14 or 42 days after vaccination were completely protected from clinical signs, and did not become viremic [124]. In other studies with various serotypes, vaccination resulted in absent or milder clinical signs in vaccinated pigs than controls after challenge at 3 weeks or later, and some pigs seem to have been completely protected from viremia, with little or no evidence of virus replication [90;123;129-131]. In one early study, two doses were required to completely protect most pigs [129]. The inclusion of the NSP protein 2B appears to increase the efficacy of Ad5-vectored FMD vaccines. In a recent study using this construct, vaccinated pigs had no clinical signs and no viremia, and did not shed virus in nasal secretions when challenged at 21 days by intradermal inoculation [90]. All pigs had neutralizing titers by 7 days, which peaked at 14 days. A bivalent vaccine that contained both A24 Cruzeiro and O1 Campos was less successful. Although pigs produced neutralizing antibodies against both serotypes, the titer were much lower than those induced by conventional commercial FMD vaccines or monovalent Ad5-A24 vaccine in previous experiments [125]. Ad5-vectored FMD vaccines have also shown promise in cattle. Cattle challenged 7 days after vaccination were protected from disseminated disease and viremia, although there was evidence of some virus replication [132]. Some vaccinated cattle are stated to have detectable virus neutralization titers as early as day 4, with all cattle seropositive by 1 week after immunization [7]. DOI studies had not been done as of 2007 [7].

6.7.2 Adenovirus Vectors and Safety
The Ad5 vector is based on a human adenovirus, and real or perceived public concerns about this vector may need to be addressed. The Ad5-FMD vaccine is classified as a BL-2 agent [7]. The construct lacks three regions of the adenovirus genome necessary for virus replication, and two independent recombination events would have to occur in a single genome to generate a replication-competent adenovirus [7]. This is predicted to be extremely rare; however, the exact rate is unknown. As of 2007, there had been no adverse reactions in any clinical trials, when the vaccine was used without adjuvants, and vaccinated cattle do not seem to shed the vaccine virus [7].
Adenoviruses are widespread among vertebrates including mammals, birds and reptiles. Adenovirus 5, which infects humans, causes mild, self-limiting illness or inapparent infections in immunocompetent individuals [133]. In children, it is an important cause of mild respiratory disease with cold-like symptoms. Conjunctivitis has also been reported after experimental inoculation of the eye in human volunteers. In people, live non-attenuated adenoviruses are given orally as vaccines against adenovirus-mediated respiratory disease [133]. Vaccines containing human adenoviruses 4, 7 and 21 have been used for this purpose for many years, and have an excellent safety record. Live Ad5 vaccines have also been tested by enteric administration, without adverse effects.

In contrast, there are some concerns about the parenteral use of Ad5 vectors in human cancer treatment and gene-therapy trials, especially when they are administered intravenously. 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 [134]. When given by hepatic artery infusion or intravenously, mild to moderate flu-like symptoms have been reported [134]. Some constructs injected directly into the blood had no dose-limiting toxicities; others resulted in transient dose-limiting cardiac output suppression and hypotension at the highest levels [134]. Adenovirus constructs have been used safely in some gene therapy trials; however, in 1999, a patient with ornithine transcarbamylase deficiency died after an intravenous injection of a high dose of replication-defective Ad vector expressing ornithine transcarbamylase, in a Phase I clinical trial [133]. This death, which was widely publicized, is unrelated to Ad5 use as a vaccine vector; however, it may be linked to concerns about adenovirus vectors, in general, with regard to popular perception. The apparent cause of death was a cytokine cascade leading to shock with disseminated intravascular coagulation, acute respiratory distress and multiorgan failure. Adenoviruses are very effective inducers of interferon and innate immune responses, and gene therapy can result in unexpected adverse effects from these responses [128]. When Ad-based gene transfer vectors are given by intravenous administration, the amounts are much higher than would normally be found in an adenovirus infection. This patient also had elevated blood ammonia levels before treatment, which may have contributed to metabolic stress [134].

6.7.3 Other Experimental Vaccines and Approaches

A wide variety of other experimental approaches to FMD vaccination have been attempted. No vaccines produced from these investigations are close to licensing.

Subunit vaccines based on FMDV proteins and peptides, and DNA vaccines for FMD have been investigated, but they have been much less immunogenic than conventional FMD vaccines [89]. Capsid-based peptide vaccines have been tested in rodent models, and were protective in some cases [7]. However, they have not been consistently protective in cattle and pigs. Peptide vaccines also induce narrow immune responses, and viral variants can evade the immune response if a limited subset of epitopes is used [7;95]. For this reason, these vaccines might provide selection pressure for the evolution of FMDV variants.

Virus-like particles (VLPs) are formed by the self-assembly of capsid proteins. Chimeric VLPs that expose the foreign antigen on their surface have also been created (e.g., yeast-derived VLP experimental FMD vaccine, baculovirus-derived VLP experimental FMD vaccines, and hepatitis B virus-derived constructs) [7]. Most VLP-based FMD vaccines have not been tested for efficacy in cattle or swine [7]. Those that were tested were partially protective.

A cDNA-derived “killed vaccine platform” (cFMDV) for the production of a marker inactivated vaccine has been created [7]. This construct uses leader-deleted foot and mouth disease virus genome, and can express capsids of different serotypes without needing to adapt strains to cell culture. It showed complete attenuation in cattle in a pilot study.
Various gene-deleted approaches have also been investigated. Targeted deletion of the FMDV Lpro gene produced an attenuated vaccine that resulted in good neutralizing titers; however, this vaccine was not fully protective [89]. A recombinant FMDV with a deleted RGD receptor binding site, which the virus uses to enter the cell, has also been made [89]. This construct was protective, but there is a risk that FMDV variants might enter the cell using other receptors [89].

Replication-competent or replication-defective nonhuman adenoviral vectors (e.g., bovine adenovirus serotype 3, porcine adenovirus serotype 3, porcine adenovirus serotype 5) are being considered in vaccines for other diseases. There has been no research reported on these constructs with FMDV [7].

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.

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 within days. In vitro serological tests (the two dimensional virus neutralization test, ELISA and complement fixation) can also be used for vaccine matching. The ‘r’ value indicates the closeness of the match in serological tests. The r values that correlate with a good match have been published for each test. Disadvantages to in vitro serological tests are that variation between batches of antisera can cause inconsistent results, VNT and ELISA results may not agree, and serological tests alone cannot account for differences in vaccine potency. The expected percentage of protection (EPP) test can be used for both matching and potency testing. This test estimates protection based on antibody titers in cattle, using tables that correlate these titers with the protection induced by a specific vaccine. Correlation tables are not available for all vaccines.

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 improve the breadth of antigenic cover, but immunity develops more slowly than if a single dose of a highly potent vaccine is used. 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 PD50 value and PGP tests), indirect tests such as serological assays (e.g., VNT or ELISA), and the EPP test. Each test has advantages and disadvantages. Due to the inherent variability in some tests, vaccines with the same measured potencies may provide different levels of protection. The antigen load can be used as an indirect indication of potency, if certain conditions are met, but validation has been difficult to achieve. 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.
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 [13]. Challenge studies can determine both the potency of the vaccine and its cross-reactivity with the field strain [13]. However, these studies require the use of live virus and facilities for Containment Group 4 pathogens. They are also slow and expensive; it takes approximately 1 month to test existing vaccines against the field strain by this method [13]. Because a decision for emergency vaccination must often be made within days, this is often impractical [13].

In vitro alternatives to animal challenge tests can also be used. 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 [7;13].

In vivo protection from FMDV is correlated with antibody titers, and serological tests can be used for vaccine matching [116:135-137]. Tests that can be used include virus neutralization (the two dimensional neutralization test), ELISA and complement fixation [13]. In vitro serological tests can generate results rapidly and do not require animal testing. ELISA tests do not require the use of live virus. A disadvantage to in vitro serological tests is that variation between the batches of antisera used can cause inconsistent results [13]. There can also be discrepancies between VNT and ELISA results. An additional disadvantage 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 isolates must have been serotyped and adapted to grow in cell culture [13]. At least two isolates should be tested if possible. The selection of potential vaccine strains should be based on the serotype of the field virus, its region of origin and any other information on its characteristics. Because serological assays have low repeatability, all tests should be repeated; confidence in the relatedness is related to the number of times the test is done [13]. (For details, refer to the current OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals.) If the results of serological matching are inconsistent, it should be determined whether antigenic differences are present or the inconsistency is an artifact of testing. The availability of sera for matching to particular vaccine strains may limit testing by this method.

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 [13]. Two-way testing (r2) would also
match using an antisera against the field isolate. The most relevant test for protection in the animal may be virus neutralization [13]. However, non-neutralizing antibodies may also be protective, and the ELISA is quick and requires smaller amounts of post-vaccination serum, which is often limited in supply [13]. The OIE suggests using the ELISA and CFT to screen, and VNT or the expected percentage of protection (EPP) method (see below) for more definitive results.

According to the OIE, if matching is done by ELISA and $r_1$ [13] is:
- 0.4–1.0: The field and vaccine strains are closely related. If the vaccine strain is potent, it is likely to be protective.
- 0.2–0.39: The field isolate and vaccine strain are antigenically related. The vaccine strain might be suitable, if there is no better match and the vaccine is potent. If possible, the animals should be vaccinated more than once.
- <0.2: The field isolate and vaccine strain are distantly related, and the vaccine is unlikely to be protective.

For vaccine matching by virus neutralization (the two-dimensional neutralization test), $r_1$ values greater than 0.3 suggest that the vaccine strain is likely to be protective, and values less than 0.3 indicate that protection is unlikely [13].

If matching is by complement fixation, $r_1$ values greater than 0.25 suggest that the vaccine is likely to be protective [13].

The expected percentage of protection (EPP) test is an estimate of the probability that cattle will be protected against 10,000 infective doses after a single or boosted (single boost) vaccination [13;137]. 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 [137]. Correlation tables are not available for all vaccines [13].

### 7.2 Vaccine Potency

Higher potency vaccines result in a faster onset of immunity and less virus shedding [13;59;137]. They are also thought to provide better protection against heterologous strains of FMDV, although there are currently few published studies to support this concept [13;59;118;137]. Initial studies reported at a meeting for representatives of vaccine banks suggest that this effect occurs with some but not all strains [118]. Boosters can also be used to increase vaccine efficacy and improve the breadth of antigenic cover, but immunity develops more slowly than if a single dose of a highly potent vaccine is used [13;137].

Higher antigen levels usually indicate that the vaccine is more potent [59]. However, the amount of antigen needed to reach a specific level of potency varies with the strain [118]. 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 [138]. 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 [139]. 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 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 [59]. 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 [13]. The groups are vaccinated with a full dose of vaccine or two different partial doses (e.g., ¼ and 1/16 dose). Two additional animals are nonvaccinated controls. All animals are challenged with 10,000 BID$_{50}$ (50% bovine infectious dose) 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 [59;137]. It is impossible to distinguish vaccines with a PD$_{50}$ of 3, 6 or 10 based on the outcome of a single potency trial [13;119;140]. One study found that FMD vaccines with the same PD$_{50}$ did not necessarily share a common level of protection [141]. 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 [142].

An alternative potency test, which is used in South America, is also accepted by the OIE [13]. 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$_{50}$ (50% bovine infectious dose), 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 [143]. This study also indicated that more potent vaccines produce more consistent results in the PGP test [143]. To increase the statistical power of the PGP test, it has been suggested that additional animals could be included [143]. 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 [143].

The potency test for pigs is a modification of the cattle PD$_{50}$ or PGP test [13]. Pigs are usually challenged on day 28 or later in the PD$_{50}$ test, although this interval can be decreased to 3 weeks with some formulations. Potency tests in other species such as sheep, goats or buffalo are 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 [13]. Because African buffalo, Asian water buffalo and sheep 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 [13].

Indirect tests such as the measurement of FMDV-specific titers by ELISA or virus neutralization, or serum-protecting antibodies in suckling mice, can be used to measure potency if there is a satisfactory correlation between the test results and the potency test in cattle [13]. Advantages of using serological testing include a decreased risk that live virus will escape, and benefits to the welfare of the experimental animals [141]. The test results are also more precise because serological tests can be quantified using a continuous scale, unlike challenge experiments [141]. The antigen load can be used as an indirect indication of potency, if there is a known correlation been the amount of antigen, serological response and protection against challenge, and an alternative test that measures antibody responses after vaccination was satisfactory [13]. However, validation of the antigen load as a potency test has been difficult to
achieve [119]. A disadvantage of indirect tests, compared to in vivo testing in cattle, is that cellular immunity is not measured [119].

The EPP test, which is used for vaccine matching, is a serologic test that can also be employed for potency testing [144]. In Argentina, this assay (using ELISA titers) has partly replaced the PGP test for cattle ([145] cited in [144]). The PGP challenge test is still used during vaccine licensing, or when new strains are included in a vaccine. A recent study reported that the EPP test was less variable than the PGP assay for the same A24 Cruzeiro vaccine [144]. A high degree of concordance was reported between mean EPP values from virus neutralization or ELISA testing and the PGP test [144]. The EPP based on VNT was more variable than EPP based on ELISA, and falsely rejected the vaccine batch on one of 10 occasions [144].

7.3 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 [146]. 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 [146]. This was a concern with early formaldehyde-inactivated FMD vaccines, and resulting in switching to aziridine inactivation in the 1970s [7;13;87]. There are no reports of failed inactivation with modern FMD vaccines when good manufacturing practices and quality control are practiced [7]. However, these measures are critical. Type C outbreaks in Kenya [147] and FMDV viruses circulating in China and eastern Russia in 2005 [119] 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 [146]. Live genetically modified organisms or vectored vaccines usually have higher-risk profiles. Adjuvants and other vaccine ingredients may cause local or systemic reactions in some animals [146]. Granulomas, abscesses, inflammation and necrosis or fibrosis may occur at the injection site. Fever, lethargy, anorexia, arthritis, soreness, decreased milk yield and allergic reactions are also possible. 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 [39]. This reaction is thought to be a hypersensitivity reaction. Post-vaccination allergic reactions were also reported in Israel in 2001 [148]. Necrotic dermatitis and other adverse reactions were seen in a dairy cattle herd 8 days after the annual FMD vaccination. The reactions occurred in 11.3% of the heifers, in 10% of the first-lactation cows and in 14.6% of the adult cows. The average loss of milk production for an affected cow on this farm was 21.5% per day, for seven days. Contamination of vaccines by extraneous pathogens could also cause morbidity or mortality [146]. Consideration should be given to the possibility of interactions with other vaccines [146].

Risks to people who administer or contact the vaccine should also be assessed. There is no evidence that the antigens in inactivated FMD viruses are a safety hazard for humans [149]. However, local reactions from oil adjuvants or other ingredients should be addressed in label warnings [146].

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 [146]. Other vaccine components such as adjuvants and excipients must also be considered in the safety evaluation, and may
require withdrawal periods [146]. Prior experiences with these components in other vaccines should be considered [146]. 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 Ad5-vectorized FMD vaccine 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 [149].

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. [7]. They recommended that the North American FMD Vaccine Bank stockpile monovalent and multivalent, 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. In the long term, the development and licensing of human adenovirus 5 vectorized-FMD serotype- and subtype-specific vaccines should be contracted. FMDCWG also recommended that the U.S. stockpile (e.g., establish contracts to deliver) commercial 3ABC test kits (Cedi-diagnostics) 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 [7]. The Ceditest ELISA is fully validated in cattle, and confirmatory testing has been completed for its use with Ad5-vectorized FMD vaccines [7]. An additional advantage to this test is that it is a blocking ELISA and can be used with any species [99]. Several other NSP tests are indirect ELISAs, and some components of these tests are species-specific [99].

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 ([150] cited in [151]). Movement controls and quarantines also decrease transmission between farms [91;151]. For these reasons, Rh values are expected to be lower than R0 values [151].

Experimental studies on transmission suggest that:
- Vaccination can reduce FMDV transmission in cattle and sheep [31;81;82;152;153]. In some cases,
immunization with a potent vaccine may decrease the estimated value of R to less than 1 [31;81;82]. Some vaccines may be more effective than others [153]. In ruminants, vaccination reduces virus shedding in oropharyngeal secretions and milk, as well as decreasing viremia [31;40;79-82;84;85;119;153-158]. Occasionally, vaccines can completely prevent virus shedding in some individual cattle or sheep in an experiment [82;139;158]. 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 [142].

- Vaccination reduces virus shedding in pigs in some experiments [40;62;159-162]. Some experimental and field studies have also reported that vaccination can decrease virus transmission to contacts [159-161-165]. 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 [83]. 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 [142].

- Some studies suggest that vaccination is more effective in reducing virus shedding and transmission when the interval between vaccination and challenge is longer [71;152;159;161;162]. (See also “onset of immunity” below.)

Vaccines may perform better in experimental animals than in the field [151]. 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 and Virus Shedding in Cattle

In lactating dairy cows, a single vaccination with an oil adjuvanted type O vaccine (PD50 of approximately 9) appeared to be capable of halting virus transmission when the animals were challenged after 2 weeks [82]. 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 $\infty (1.3; \infty)$, 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 [81]. 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 [81].

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 [152]. 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 [79;155;156]. 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 [157]. 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 [71]. Some experiments have also reported that conventional FMD vaccines can decrease virus shedding in cattle [40;119;154].

10.2 Transmission Studies and Virus Shedding in Sheep

In a study of virus transmission among lambs, vaccination with an oil adjuvanted vaccine (PD$_{50}$ of at least 6) did not eliminate virus shedding when the animals were challenged after 2 weeks, but it significantly decreased both the amount of virus shed and the duration of shedding [31]. 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 [31]. 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 C1 Oberbayern vaccine, but animals vaccinated with oil formulated Asia 1 India vaccine transmitted a homologous virus to susceptible contacts [153]. 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 [158]. 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 in other studies [80;84;85;153], and vaccines with higher antigen payloads decreased virus replication more than vaccines with smaller amounts of antigen [80].

10.3 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 [161;162]. 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 [161]. A meta-analysis of this and other experiments from this group [166] 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 [163]. 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 [160]. However, Orsel et al reported that, with a severe challenge, vaccination did not reduce virus shedding compared to nonvaccinated animals [83]. The R value was higher in nonvaccinated pigs (∞ [1.3; ∞]) than vaccinated pigs (2.42 [0.9; 6.9]), but the difference was not statistically significant. The transmission rate (β) was, however, significantly lower between vaccinated pigs (6.84 day⁻¹) than between nonvaccinated pigs (0.66 day⁻¹), suggesting that immunization might slow virus spread. 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 [62]. Other studies have reported that vaccination decreases virus shedding in aerosols and/or reduces transmission [40;159]. 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 [159].

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 [164]. 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 with O/Taiwan/97 in Taiwan [165]. These studies suggest that vaccination might have a significant effect on virus transmission under field conditions.

### 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 FMD vaccine), several outbreaks occurred 2-9 days after vaccination was performed on a farm [59;95;167]. 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 [7;13;25;85]. Most early vaccine studies and studies by the manufacturer for licensing have measured vaccine efficacy by evaluating clinical signs. However, clinical protection does not necessarily 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 (see tables below) can be made:

- **Vaccination is more effective when the time between vaccination and exposure is longer [62;71;79;152;155;157;159].**
- **Animals that are protected from clinical signs may still shed virus or transmit the infection [31;62;80;81;139;152;153;155;157-159].** However, some vaccines can significantly reduce virus shedding and decrease or prevent transmission [31;62;71;80-82;84;152-155;157-161;168].
- **Vaccination can sometimes protect cattle from clinical signs as soon as 4-5 days [79;152;168].** One study suggests that virus shedding may be decreased at this time [71]. By 14 days, other studies also...
suggest that virus shedding and transmission are reduced in this species [81;82;152]. 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 [155;157].

- In sheep, vaccination may decrease virus shedding and/or transmission as early as 3-7 days [85;153;158;169]. Other challenge studies conducted 2 or 3 weeks after vaccination also report decreased shedding and/or transmission [31;80;84]. 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 [85;153]. Madhanmohan et al. reported fever but no vesicles in some animals challenged at 21 days [84].

- 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 [159;160;168]; 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 [79]. 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 [62]. 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 [83]. 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 [159-161]. 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 [161;166].

- 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 [137]. All cattle challenged with homologous virus were protected from clinical signs in this experiment.

- 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 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, emergency vaccines [170]. The titers peaked in most animals at 28 days. Detectable titers of neutralizing antibodies have also been reported within 7 days in pigs vaccinated with high potency, oil adjuvanted, emergency vaccines, with titers peaking at 21-28 days [170].
## 11.1 Tables Summarizing Experimental Studies

### Table 1: Cattle

<table>
<thead>
<tr>
<th>Interval between vaccination and challenge (days)</th>
<th>Parameter</th>
<th>Effect of vaccination</th>
<th>Vaccine potency, adjuvant</th>
<th>Challenge type</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>4, 8, 12, 16 or 21</td>
<td>Clinical signs</td>
<td>Protected</td>
<td>$PD_{50} = 41; \text{oil or Al(OH)}_3$</td>
<td>Aerosols from pigs for 1 hour</td>
<td>Doel et al., 1994</td>
</tr>
<tr>
<td>4 or 7</td>
<td>Clinical signs</td>
<td>Protected</td>
<td>$PD_{50}$ of 19; Al(OH)$_3$</td>
<td>Aerosols from pigs for 1 hour</td>
<td>Donaldson and Kitching, 1989</td>
</tr>
<tr>
<td>4 or 7</td>
<td>Virus transmission</td>
<td>Transmitted</td>
<td>$PD_{50}$ of 19; Al(OH)$_3$</td>
<td>Aerosols from pigs for 1 hour</td>
<td>Donaldson and Kitching, 1989</td>
</tr>
<tr>
<td>14</td>
<td>Clinical signs</td>
<td>Protected</td>
<td>$PD_{50}$ of 19; Al(OH)$_3$</td>
<td>Aerosols from pigs for 1 hour</td>
<td>Donaldson and Kitching, 1989</td>
</tr>
<tr>
<td>14</td>
<td>Virus transmission</td>
<td>Sometimes transmitted, some virus shedding</td>
<td>$PD_{50}$ of 19; Al(OH)$_3$</td>
<td>Aerosols from pigs for 1 hour</td>
<td>Donaldson and Kitching, 1989</td>
</tr>
<tr>
<td>21</td>
<td>Clinical signs</td>
<td>Protected</td>
<td>$PD_{50}$ of 19; Al(OH)$_3$</td>
<td>Aerosols from pigs for 1 hour</td>
<td>Donaldson and Kitching, 1989</td>
</tr>
<tr>
<td>21</td>
<td>Virus transmission</td>
<td>Did not transmit, but some virus shedding</td>
<td>$PD_{50}$ of 19; Al(OH)$_3$</td>
<td>Aerosols from pigs for 1 hour</td>
<td>Donaldson and Kitching, 1989</td>
</tr>
<tr>
<td>4</td>
<td>Clinical signs</td>
<td>Protected</td>
<td>oil</td>
<td>Direct contact with infected cattle</td>
<td>Graves et al., 1968</td>
</tr>
<tr>
<td>5</td>
<td>Clinical signs</td>
<td>3 of 4 cattle protected</td>
<td>oil</td>
<td>Direct contact with infected cattle</td>
<td>Graves et al., 1968</td>
</tr>
<tr>
<td>7, 10 or 14</td>
<td>Clinical signs</td>
<td>Protected</td>
<td>oil</td>
<td>Direct contact with infected cattle</td>
<td>Graves et al., 1968</td>
</tr>
<tr>
<td>4</td>
<td>Clinical signs</td>
<td>Decreased clinical signs</td>
<td>$PD_{50}$ of 3; oil</td>
<td>Needle inoculation</td>
<td>Golde et al., 2005</td>
</tr>
<tr>
<td>4</td>
<td>Virus shedding</td>
<td>Decreased virus shedding, no viremia</td>
<td>$PD_{50}$ of 3; oil</td>
<td>Needle inoculation</td>
<td>Golde et al., 2005</td>
</tr>
<tr>
<td>7, 14 or 21</td>
<td>Clinical signs</td>
<td>Protected</td>
<td>$PD_{50}$ of 3; oil</td>
<td>Needle inoculation</td>
<td>Golde et al., 2005</td>
</tr>
<tr>
<td>10</td>
<td>Clinical signs</td>
<td>Most protected, a few had mild signs</td>
<td>$PD_{50}$ of 18 oil</td>
<td>Contact with infected cattle for 5 days, different virus of same serotype</td>
<td>Cox et al., 2007</td>
</tr>
<tr>
<td>10</td>
<td>Virus shedding</td>
<td>Virus shedding only reduced during earliest period after infection</td>
<td>$PD_{50}$ of 18 oil</td>
<td>Contact with infected cattle for 5 days, different virus of same serotype</td>
<td>Cox et al., 2007</td>
</tr>
</tbody>
</table>
### Table 1: Cattle (cont’d)

<table>
<thead>
<tr>
<th>Interval between vaccination and challenge (days)</th>
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</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>Virus shedding</td>
<td>Possibly decreased virus shedding during earliest period after infection, compared to 1x dose of vaccine; no effect on carriers</td>
<td>10x dose of vaccine above</td>
<td>Contact with infected cattle for 5 days, different virus of same serotype</td>
<td>Cox et al., 2007</td>
</tr>
<tr>
<td>14</td>
<td>Clinical signs (adult dairy cattle)</td>
<td>Protected</td>
<td>PD(_{50}) of 9, oil</td>
<td>Intranasal inoculation</td>
<td>Orsel, de Jong, et al., 2007¹.</td>
</tr>
<tr>
<td>14</td>
<td>Virus transmission</td>
<td>No transmission, no virus shedding</td>
<td>PD(_{50}) of 9, oil</td>
<td>Intranasal inoculation</td>
<td>Orsel, de Jong, et al., 2007¹.</td>
</tr>
<tr>
<td>14</td>
<td>Clinical signs (calves)</td>
<td>Protected</td>
<td>PD(_{50}) ≥ 32</td>
<td>Injection</td>
<td>Brehm et al., 2008</td>
</tr>
<tr>
<td>21</td>
<td>Clinical signs</td>
<td>Protected</td>
<td>PD(_{50}) ≥ 32 to homologous virus; PD50 of at least 6 to challenge virus</td>
<td>Injection; different virus of same serotype; r values &lt; 0.3.</td>
<td>Brehm et al., 2008</td>
</tr>
<tr>
<td>21</td>
<td>Clinical signs</td>
<td>Protected</td>
<td>PD(_{50}) of 18, oil</td>
<td>Contact with infected cattle for 5 days, different virus of same serotype</td>
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</thead>
<tbody>
<tr>
<td>21</td>
<td>Virus shedding, infection</td>
<td>Virus shedding reduced, 17/20 cattle became infected, 45% carriers by RNA, difficult to isolate virus from carriers</td>
<td>PD$_{50}$ of 18, oil</td>
<td>Contact with infected cattle for 5 days, different virus of same serotype</td>
<td>Cox et al., 2005</td>
</tr>
<tr>
<td>21</td>
<td>Neutralizing antibodies, number of carriers</td>
<td>Higher titers; fewer carriers (not statistically significant)</td>
<td>10x dose of vaccine above</td>
<td>Contact with infected cattle for 5 days, different virus of same serotype</td>
<td>Cox et al., 2006</td>
</tr>
<tr>
<td>28-140</td>
<td>Clinical signs</td>
<td>Fewer had clinical signs, signs were less severe</td>
<td>Oil; crude vaccine made for experiment</td>
<td>Intranasal inoculation</td>
<td>McVicar and Sutmoller, 1976</td>
</tr>
<tr>
<td>28-140</td>
<td>Virus shedding</td>
<td>Decreased virus shedding, no viremia</td>
<td>Oil; crude vaccine made for experiment</td>
<td>Intranasal inoculation</td>
<td>McVicar and Sutmoller, 1976</td>
</tr>
<tr>
<td>28</td>
<td>Clinical signs</td>
<td>No effect</td>
<td>oil, PD$_{50}$ of 1.8 (lower than OIE standards for conventional vaccine); 1/16th, 1/4 or full dose</td>
<td>Injection</td>
<td>Moonen et al., 2004</td>
</tr>
<tr>
<td>179 (6 months)</td>
<td>Clinical signs</td>
<td>Protected</td>
<td>High potency (PD$_{50}$ unavailable); Oil</td>
<td>Injection</td>
<td>Cox et al., 2010</td>
</tr>
</tbody>
</table>

Table 2: Sheep

<table>
<thead>
<tr>
<th>Interval between vaccination and challenge (days)</th>
<th>Parameter</th>
<th>Effect of vaccination</th>
<th>Vaccine potency, adjuvant</th>
<th>Challenge type</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>3, 4, 6 or 10</td>
<td>Clinical signs</td>
<td>No controls or vaccinated sheep had vesicles, some controls and no vaccinated sheep had fever</td>
<td>PD$_{50}$ of 41; oil or Al(OH)3</td>
<td>Aerosols from pigs, 2 hours</td>
<td>Cox et al., 1999</td>
</tr>
<tr>
<td>3, 4, 6 or 10</td>
<td>Virus shedding</td>
<td>Fewer shed virus in nasal secretions</td>
<td>PD$_{50}$ of 41; oil or Al(OH)3</td>
<td>Aerosols from pigs, 2 hours</td>
<td>Cox et al., 1999</td>
</tr>
</tbody>
</table>
### Table 2: Sheep (cont’d)

<table>
<thead>
<tr>
<th>Interval between vaccination and challenge (days)</th>
<th>Parameter</th>
<th>Effect of vaccination</th>
<th>Vaccine potency, adjuvant</th>
<th>Challenge type</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>3, 4, 6 or 10</td>
<td>Transmission</td>
<td>Virus transmitted to contacts</td>
<td>PD&lt;sub&gt;50&lt;/sub&gt; of 61; oil</td>
<td>Aerosols from pigs, 4 hours</td>
<td>Cox et al., 1999</td>
</tr>
<tr>
<td>4, 5, 7 or 11</td>
<td>Transmission</td>
<td>No transmission to contacts, decreased virus shedding</td>
<td>PD&lt;sub&gt;50&lt;/sub&gt; ≥ 112; Al(OH)3</td>
<td>Aerosols from pigs, 4 hours</td>
<td>Cox et al., 1999</td>
</tr>
<tr>
<td>4 or 10</td>
<td>Clinical signs</td>
<td>Protected from vesicles, some had fever (milder than controls)</td>
<td>PD&lt;sub&gt;50&lt;/sub&gt; of 18; oil</td>
<td>Aerosols from pigs, 9 hours</td>
<td>Parida et al., 2008</td>
</tr>
<tr>
<td>4 or 10</td>
<td>Virus shedding</td>
<td>Decreased</td>
<td>PD&lt;sub&gt;50&lt;/sub&gt; of 18; oil</td>
<td>Aerosols from pigs, 9 hours</td>
<td>Parida et al., 2008</td>
</tr>
<tr>
<td>7</td>
<td>Clinical signs</td>
<td>Almost always protected</td>
<td>conventional vaccine, given at 3x or 6x; vaccine stored for 10 months before use</td>
<td>Aerosols from pigs, 2 hours</td>
<td>Gibson et al., 1984</td>
</tr>
<tr>
<td>7</td>
<td>Virus shedding</td>
<td>Virus shedding decreased with 6x dose</td>
<td>conventional vaccine, given at 3x or 6x; vaccine stored for 10 months before use</td>
<td>Aerosols from pigs, 2 hours</td>
<td>Gibson et al., 1984</td>
</tr>
<tr>
<td>7, 14, 21 or 35</td>
<td>Clinical signs</td>
<td>Protected, except fever in some</td>
<td>oil</td>
<td>Contact with sheep, 24 hours</td>
<td>Madhanmohan, Nagendra Kumar, Srinivasan, 2010</td>
</tr>
<tr>
<td>7, 14, 21 or 35</td>
<td>Virus shedding</td>
<td>Decreased in nasal secretions by 100-fold, decreased in oropharynx by 1000-fold, no viremia</td>
<td>oil</td>
<td>Contact with sheep, 24 hours</td>
<td>Madhanmohan, Nagendra Kumar, Srinivasan, 2010</td>
</tr>
<tr>
<td>14</td>
<td>Clinical signs</td>
<td>Protected</td>
<td>PD&lt;sub&gt;50&lt;/sub&gt; ≥ 6; oil</td>
<td>Intranasal inoculation</td>
<td>Orsel, Dekker et al., 2007</td>
</tr>
<tr>
<td>14</td>
<td>Virus shedding</td>
<td>Decreased</td>
<td>PD&lt;sub&gt;50&lt;/sub&gt; ≥ 6; oil</td>
<td>Intranasal inoculation</td>
<td>Orsel, Dekker et al., 2007</td>
</tr>
<tr>
<td>14</td>
<td>Clinical signs</td>
<td>Protected</td>
<td>full dose, 1/10 or 1/40 dose of a high potency (PD&lt;sub&gt;50&lt;/sub&gt;=41); Al(OH)3</td>
<td>Aerosols from pigs, 4 hours</td>
<td>Barnett et al., 2004</td>
</tr>
</tbody>
</table>
### Table 2: Sheep (cont’d)

<table>
<thead>
<tr>
<th>Interval between vaccination and challenge (days)</th>
<th>Parameter</th>
<th>Effect of vaccination</th>
<th>Vaccine potency, adjuvant</th>
<th>Challenge type</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>14</td>
<td>Virus shedding</td>
<td>Decreased; greatest effect with highest dose vaccine</td>
<td>full dose, 1/10 or 1/40 dose of a high potency (PD$_{50}$=41); Al(OH)$_3$</td>
<td>Aerosols from pigs, 4 hours</td>
<td>Barnett et al., 2004</td>
</tr>
<tr>
<td>21</td>
<td>Clinical signs</td>
<td>Protected from vesicles, fever in some</td>
<td>oil, single dose, either 1x or 4x antigen</td>
<td>Injection</td>
<td>Madhanmohan, Nagendrakumar, Narasu, Srinivasan, 2010</td>
</tr>
<tr>
<td>21</td>
<td>Virus shedding</td>
<td>Sheding decreased, no viremia</td>
<td>oil, single dose, either 1x or 4x antigen</td>
<td>Injection</td>
<td>Madhanmohan, Nagendrakumar, Narasu, Srinivasan, 2010</td>
</tr>
<tr>
<td>28</td>
<td>Clinical signs</td>
<td>Protected from vesicles, fever in some</td>
<td>oil, one booster, either 1x or 4x antigen</td>
<td>Injection</td>
<td>Madhanmohan, Nagendrakumar, Narasu, Srinivasan, 2010</td>
</tr>
<tr>
<td>28</td>
<td>Virus shedding</td>
<td>Sheding decreased, no viremia</td>
<td>oil, one booster, either 1x or 4x antigen</td>
<td>Injection</td>
<td>Madhanmohan, Nagendrakumar, Narasu, Srinivasan, 2010</td>
</tr>
</tbody>
</table>

### Table 3: Goats

<table>
<thead>
<tr>
<th>Interval between vaccination and challenge (days)</th>
<th>Parameter</th>
<th>Effect of vaccination</th>
<th>Vaccine potency, adjuvant</th>
<th>Challenge type</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>7, 14, 21 or 35</td>
<td>Clinical signs</td>
<td>Protected, except fever in some</td>
<td>oil</td>
<td>Contact with goats, 24 hours</td>
<td>Madhanmohan, Nagendrakumar, Srinivasan, 2010</td>
</tr>
<tr>
<td>7, 14, 21 or 35</td>
<td>Virus shedding</td>
<td>Decreased in nasal secretions by 100-fold, decreased in oropharynx by 1000-fold, no viremia</td>
<td>oil</td>
<td>Contact with goats, 24 hours</td>
<td>Madhanmohan, Nagendrakumar, Srinivasan, 2010</td>
</tr>
<tr>
<td>Interval between vaccination and challenge (days)</td>
<td>Parameter</td>
<td>Effect of vaccination</td>
<td>Vaccine potency, adjuvant</td>
<td>Challenge type</td>
<td>Reference</td>
</tr>
<tr>
<td>-------------------------------------------------</td>
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</tr>
<tr>
<td>2 or 3</td>
<td>Clinical signs</td>
<td>Not protected</td>
<td>PD$_{50} &gt; 112$, oil</td>
<td>Aerosols from pigs, 4 hours</td>
<td>Salt et al., 1998</td>
</tr>
<tr>
<td>2 or 3</td>
<td>Virus transmission</td>
<td>Became viremic, transmitted virus</td>
<td>PD$_{50} &gt; 112$, oil</td>
<td>Aerosols from pigs, 4 hours</td>
<td>Salt et al., 1998</td>
</tr>
<tr>
<td>4</td>
<td>Clinical signs</td>
<td>Protected initially; later developed clinical signs from exposure to nonvaccinated contacts</td>
<td>PD$_{50} &gt; 112$, oil</td>
<td>Aerosols from pigs, 4 hours</td>
<td>Salt et al., 1998</td>
</tr>
<tr>
<td>4</td>
<td>Virus transmission</td>
<td>Virus transmitted, but decreased virus shedding</td>
<td>PD$_{50} &gt; 112$, oil</td>
<td>Aerosols from pigs, 4 hours</td>
<td>Salt et al., 1998</td>
</tr>
<tr>
<td>5</td>
<td>Clinical signs</td>
<td>One of 3 pigs protected</td>
<td>PD$_{50} &gt; 112$, oil</td>
<td>Aerosols from pigs, 4 hours</td>
<td>Salt et al., 1998</td>
</tr>
<tr>
<td>5</td>
<td>Virus transmission</td>
<td>Virus transmitted</td>
<td>PD$_{50} &gt; 112$, oil</td>
<td>Aerosols from pigs, 4 hours</td>
<td>Salt et al., 1998</td>
</tr>
<tr>
<td>7</td>
<td>Clinical signs</td>
<td>Protected</td>
<td>PD$_{50} &gt; 112$, oil</td>
<td>Aerosols from pigs, 4 hours</td>
<td>Salt et al., 1998</td>
</tr>
<tr>
<td>7</td>
<td>Virus transmission</td>
<td>Not transmitted, virus shedding decreased</td>
<td>PD$_{50} &gt; 112$, oil</td>
<td>Aerosols from pigs, 4 hours</td>
<td>Salt et al., 1998</td>
</tr>
<tr>
<td>4, 8, 12, 16 or 21</td>
<td>Clinical signs</td>
<td>Protected</td>
<td>PD$_{50} &gt; 112$, oil</td>
<td>Aerosols from pigs, 1 hour</td>
<td>Salt et al., 1998</td>
</tr>
<tr>
<td>3</td>
<td>Clinical signs</td>
<td>3 of 4 pigs protected</td>
<td>oil</td>
<td>Direct contact with inoculated pigs</td>
<td>Graves et al., 1968</td>
</tr>
<tr>
<td>5</td>
<td>Clinical signs</td>
<td>2 of 4 pigs protected</td>
<td>oil</td>
<td>Direct contact with inoculated pigs</td>
<td>Graves et al., 1968</td>
</tr>
<tr>
<td>7</td>
<td>Clinical signs</td>
<td>Protected</td>
<td>oil</td>
<td>Direct contact with inoculated pigs</td>
<td>Graves et al., 1968</td>
</tr>
<tr>
<td>10</td>
<td>Clinical signs</td>
<td>3 of 4 pigs protected</td>
<td>oil</td>
<td>Direct contact with inoculated pigs</td>
<td>Graves et al., 1968</td>
</tr>
<tr>
<td>14</td>
<td>Clinical signs</td>
<td>Protected</td>
<td>oil</td>
<td>Direct contact with inoculated pigs</td>
<td>Graves et al., 1968</td>
</tr>
<tr>
<td>4, 5 or 6</td>
<td>Clinical signs</td>
<td>Clinical signs in some high potency, oil</td>
<td>Direct contact with pigs, 2 hours</td>
<td>Barnett, Garland et al., 2002</td>
<td></td>
</tr>
<tr>
<td>Interval between vaccination and challenge (days)</td>
<td>Parameter</td>
<td>Effect of vaccination</td>
<td>Vaccine potency, adjuvant</td>
<td>Challenge type</td>
<td>Reference</td>
</tr>
<tr>
<td>--------------------------------------------------</td>
<td>------------------</td>
<td>---------------------------------------------------------------------------------------</td>
<td>--------------------------------</td>
<td>-----------------------------------------------------</td>
<td>---------------------------------------------------------------------------</td>
</tr>
<tr>
<td>4, 5 or 6</td>
<td>Virus transmission</td>
<td>Decreased virus shedding, virus was transmitted</td>
<td>High potency, oil</td>
<td>Direct contact with pigs, 2 hours</td>
<td>Barnett, Garland et al., 2002</td>
</tr>
<tr>
<td>4-16</td>
<td>Clinical signs</td>
<td>A few animals protected; most had clinical signs</td>
<td>PD$_{50} = 41$, oil</td>
<td>Aerosols from pigs for 1 hour</td>
<td>Doel et al., 1994</td>
</tr>
<tr>
<td>21 or 28</td>
<td>Clinical signs</td>
<td>Completely protected</td>
<td>PD$_{50} = 41$, oil</td>
<td>Aerosols from pigs for 1 hour</td>
<td>Doel et al., 1994</td>
</tr>
<tr>
<td>7</td>
<td>Clinical signs</td>
<td>Most pigs not protected</td>
<td>oil</td>
<td>Continuous exposure to infected pigs</td>
<td>Eble et al., 2004</td>
</tr>
<tr>
<td>7</td>
<td>Virus transmission</td>
<td>Transmission occurred</td>
<td>oil</td>
<td>Continuous exposure to infected pigs</td>
<td>Eble et al., 2004</td>
</tr>
<tr>
<td>7</td>
<td>Virus transmission</td>
<td>Transmission and virus shedding decreased</td>
<td>oil, 4x dose of vaccine above</td>
<td>Continuous exposure to infected pigs</td>
<td>Eble et al., 2004</td>
</tr>
<tr>
<td>14</td>
<td>Clinical signs</td>
<td>No generalized lesions; localized lesions at injection site in some pigs</td>
<td>oil</td>
<td>Continuous exposure to infected pigs</td>
<td>Eble et al., 2004</td>
</tr>
<tr>
<td>14</td>
<td>Virus transmission</td>
<td>Not transmitted to contacts, virus shedding decreased</td>
<td>oil</td>
<td>Continuous exposure to infected pigs</td>
<td>Eble et al., 2004</td>
</tr>
<tr>
<td>14</td>
<td>Clinical signs</td>
<td>Not protected</td>
<td>PD$_{50} &gt; 6$, oil</td>
<td>Exposure to infected pigs</td>
<td>Orsel, de Jong, et al., 2007*</td>
</tr>
<tr>
<td>14</td>
<td>Virus shedding</td>
<td>Vaccination did not significantly reduce shedding</td>
<td>PD$_{50} &gt; 6$, oil</td>
<td>Exposure to infected pigs</td>
<td>Orsel, de Jong, et al., 2007*</td>
</tr>
<tr>
<td>14</td>
<td>Clinical signs</td>
<td>Clinical signs in some</td>
<td>PD$_{50} &gt; 6$, oil</td>
<td>Exposure to infected, vaccinated pigs</td>
<td>Orsel, de Jong, et al., 2007*</td>
</tr>
<tr>
<td>14</td>
<td>Virus transmission</td>
<td>Transmission not prevented, transmission rate decreased</td>
<td>PD$_{50} &gt; 6$, oil</td>
<td>Exposure to infected, vaccinated pigs</td>
<td>Orsel, de Jong, et al., 2007*</td>
</tr>
<tr>
<td>10</td>
<td>Clinical signs</td>
<td>81% had clinical signs, some were severe; milder clinical signs than controls</td>
<td>PD$_{50}$ of 18, oil</td>
<td>Exposure to infected pigs, 9 hours, different virus of same serotype</td>
<td>Parida, Fleming, Oh et al., 2007</td>
</tr>
</tbody>
</table>

Table 4: Pigs (cont’d)

<table>
<thead>
<tr>
<th>Interval between vaccination and challenge (days)</th>
<th>Parameter</th>
<th>Effect of vaccination</th>
<th>Vaccine potency, adjuvant</th>
<th>Challenge type</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>Virus shedding</td>
<td>Decreased, especially in pigs that were clinically protected</td>
<td>PD$_{50}$ of 18, oil</td>
<td>Exposure to infected pigs, 9 hours, different virus of same serotype</td>
<td>Parida, Fleming, Oh et al., 2007</td>
</tr>
<tr>
<td>29</td>
<td>Clinical signs</td>
<td>25% had mild clinical signs</td>
<td>PD$_{50}$ of 18, oil</td>
<td>Exposure to infected pigs, 9 hours, different virus of same serotype</td>
<td>Parida, Fleming, Oh et al., 2007</td>
</tr>
<tr>
<td>29</td>
<td>Virus shedding</td>
<td>Decreased shedding</td>
<td>PD$_{50}$ of 18, oil</td>
<td>Exposure to infected pigs, 9 hours, different virus of same serotype</td>
<td>Parida, Fleming, Oh et al., 2007</td>
</tr>
</tbody>
</table>

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. This approach has been promising in experimental studies in pigs.

Other interventions might theoretically be combined with vaccination to provide immunity early, before vaccine-induced immunity develops [95]. FMDV is very sensitive to interferon [7;171], and interferon-based protection has been promising in experimental studies in pigs. In one experiment, pigs that were inoculated with both an Ad5-vectored FMD vaccine and the porcine interferon-α gene were completely protected from clinical signs, viremia or virus shedding, when challenged after 5 days [130]. Pigs that received the IFN construct alone were completely protected from viremia and clinical signs if they were challenged after 1 or 3 days, and partially protected if challenged 5 or 7 days after or 1 day before inoculation with the construct [130]. 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 [130]. In another study, a combination of Ad5-vectored porcine IFN-γ (low or high dose) and Ad5-vectored porcine IFN-α completely protected all pigs from challenge after 1 day [172]. There was no viremia and no evidence of virus shedding in nasal secretions [172]. A high dose of Ad5-pIFN-γ alone was also protective; however, pigs inoculated with either Ad5-vectored IFN-α alone or a low dose of Ad5-pIFN-γ developed clinical signs and became viremic. Ad5-vectored porcine IFN-β was also protective against A24 Cruzeiro when animals were challenged 1 day after inoculation in another study [173]. While earlier studies used FMDV serotype A24 Cruzeiro, recently these results have been extended to two other serotypes. Ad5-vectored porcine IFN-α was shown to provide at least partial protection against FMDV isolates A24 Cruzeiro, O1
Manisa and Asia-1, when challenged 1 day after inoculation [173]. The response appeared to be dose-dependent, and complete protection was observed with higher doses.

In cattle, the clinical signs were less severe when the animals were given Ad5-vectored IFN-α; however, the animals were not completely protected [174]. Unpublished studies in cattle at PIADC reported similar results, using Ad5-vectored IFN-α given alone or in combination with an FMD vaccine [7]. Whether the dose of the construct was too low to result in sustained IFN-α levels, or other factors are involved is not known [7].

13. DURATION OF IMMUNITY
The duration of immunity (DOI) of a vaccine is an important consideration in vaccination-to-live programs [1]. Conventional FMD vaccines used for routine prophylaxis are expected to provide only 4–6 months of immunity [36]; 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 [13;42;170]. 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 [19]. After natural infections, pigs are reported to be susceptible to challenge after 3-6 months [170]. However, there are also reports of immunity lasting for several years in ruminants that were infected with FMDV ([175] cited in [176]).

There is relatively little information on the DOI for emergency (high potency) FMD vaccines; however, a few studies suggest that these vaccines may protect cattle, sheep or pigs for 6-7 months [36;112;139;170;177;178]. Challenge studies provide the most definitive evidence, 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. Cox et al. reported that pigs vaccinated with oil adjuvanted emergency FMD vaccines and challenged at 7 months were protected from clinical signs [170]. High titers to FMDV were maintained for up to 7 months in some vaccinated pigs. 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 [177]. Cattle have been protected from clinical signs when challenged 6 months after vaccination with a single dose of a high potency, oil adjuvanted serotype A vaccine [139]. 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 [139]. Cattle vaccinated with oil adjuvanted SAT vaccines also maintained high titers for at least six months ([112] reviewed in [36]). In contrast, Barnett et al. found that titers in cattle immunized with an oil adjuvanted serotype A24 Cruzeiro vaccine were decreasing by 43 days after vaccination ([178] reviewed in [36]). No studies have been published, to date, in sheep challenged more than 28 days after a single dose of an emergency vaccine [36]. Sheep vaccinated with high potency emergency FMD vaccines maintained titers for up to 6 months [36;170;177]. 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 270 days with an oil adjuvanted formulation, and for up to 180 days with an aluminum hydroxide adjuvanted vaccine [111].
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 ([179] cited in [81]). 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 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. Although 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.

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 [36] Together with other control measures, the European vaccination programs helped decrease the number of outbreaks, which suggests that vaccination reduced transmission between farms [81]. 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 an outbreak in Japan in 2010; however, only limited information has been published on this outbreak to date.) 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 [75]. 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 [180;181], and it is currently recognized as FMD-free where vaccination is not practiced [182].

15.2 Macedonia, 1996
In 1996, the same strain of FMDV also affected the former Yugoslav Republic of Macedonia [75]. 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 [180;181], and it is currently recognized as FMD-free where vaccination is not practiced [182].

15.3 Republic of Korea (South Korea), 2000
In March 2000, serotype O (Pan-Asia topotype) FMDV was reported on a dairy farm in the Kyonggi province in the Republic of Korea [183]. 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 O1 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 slaughtered either in a government indemnity program or they were sent by farmers to designated abattoirs. 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. Only dairy cattle and Korean native yellow cattle were affected in this outbreak. 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 [184].

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 [185]. 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;185]. 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 [185]. Approximately 850 herds were vaccinated. Emergency vaccination was also conducted in 175 herds around an outbreak in Kootwijkerbroek [185]. 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 [185]. The last infected herd was reported on April
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 [185]. Approximately 177,000 animals in 1931 herds were vaccinated [185]. 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 [72]. 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 [72]. Approximately 12,400 sheep, 6,900 cattle and 257 pigs were destroyed, and control measures were completed with a month [72]. The initial control strategy during an outbreak with a serotype A virus in 2001 also consisted of movement restrictions, ring vaccination and the culling of infected herds [72]. 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 [72]. 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 [72].
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 [72]. 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 [186]. 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) [187]. 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 [72;187].

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 [188;189]. 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 (O1 Campos and A24 Cruzeiro), as well as culling [188]. After September 17, movement restrictions were applied only to infected and contact herds [188]. 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 [189]. 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 [188;189]. 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 [189]. 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 [188].

Mass vaccination, twice a year, was begun in April in the affected region [188;189]. This vaccine used was quadrivalent; for the first time, it included the new A Argentina/2001 strain as well as A Argentina/2000, A24 Cruzeiro and O1 Campos. Cattle were vaccinated again in early 2002 [188]. At this time, control measures were also modified to include the slaughter of all affected and in-contact animals [189]. 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 [188]. 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 [188]. 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 [188]. 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 [73]. 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 [182].

15.6 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 [190]. 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 [191]. 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 [190]. 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 [190]. 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 [190]. 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 [165]. Neutralizing antibodies to FMDV structural proteins remained high and persisted for more than 6 months in vaccinated pigs [165]. 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

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<th>Summary</th>
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<td>In an eradication program, animals may be either “vaccinated to live” or “vaccinated to slaughter.” Both types of vaccination are expected to decrease virus transmission and decrease the short-term resources needed for carcass disposal, but will require the resources to implement, manage and maintain a vaccination, movement and permitting system for the vaccinates. 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 either stamping out or vaccination-to-slaughter.</td>
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Approaches to the application of FMD vaccination include prophylactic vaccination, emergency vaccination (which may be protective or suppressive), targeted vaccination, ring vaccination, barrier vaccination, predictive vaccination and blanket vaccination.

Because surveillance must be conducted to identify vaccinated animals that become infected, as well as to demonstrate the absence of virus transmission after the outbreak, the vaccination zone should be the smallest area necessary to control the outbreak. A variety of animal, virus and environmental factors must be considered in establishing an effective vaccination zone. Defining the size and shape of a vaccination zone in ring vaccination can be complex.

Consideration should be given to establishing a vaccination surveillance zone around the vaccination zone.

### 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 [38].

### 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) 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 [192]. 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 [100]. 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 [11;100]. 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 [11;100]. 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 [11;104].

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 [100]. In the vaccination surveillance zone, no vaccination is allowed, movements are restricted and there is enhanced disease surveillance [100].

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

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. Very few models have been published 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.

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, 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 [12;193]. 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.
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 [193]. The authors conclude that models can be valuable as predictive tools for evaluating alternative strategies in future outbreaks.

Two recent 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 [192]. 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, the average size of the epidemic decreases rapidly as the daily vaccination rate increases, and reaches a lower plateau when approximately 300,000 cattle are vaccinated per day. With longer delays, the epidemic is larger even when a very large proportion of the cattle population is eventually vaccinated. If high levels of herd immunity can eventually be established in cattle, however, the epidemic is shortened. The most important factor in the latter effect is the level of herd immunity, and it persists if the initial delays are less than one month.

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 [192]. 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 the section above). This model suggests that ring vaccination has much less effect on the length of a highly disseminated outbreak [192]. 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 that use simpler models [192].

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 [193]. This model finds that prioritizing the largest farms first increases the optimal ring size and decreases the epidemic impact. Very few models have been published 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 [194]. 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 [194].

18. MOVEMENT RESTRICTIONS AND VACCINATION

Effectively implemented movement restrictions have a significant impact on the effectiveness of a vaccination program [36]. 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 [176]. 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 [6].

19. SPECIES TO VACCINATE

Cattle are usually the most important maintenance hosts for FMD viruses [13], 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 [72;188;189]. 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 [165]. The importance of small ruminants might vary with the strain and/or epidemiological factors [29-31;54-56]. There is currently no consensus about whether small ruminants should be vaccinated during outbreaks [29;31;85]. 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 [13;25;38;85;87].

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 or results of the EPP test), 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 PD50 per cattle dose, although 6 PD50 is often preferred [13]. 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 [13;36;170]. 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 [13;36;42]. 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 [19]. If animals are difficult to access, the OIE recommends oil adjuvanted vaccines, boosted at 4 months and 1 year of age, followed by a yearly booster [13]. In FMD-endemic areas, vaccinated animals usually have time to develop and maintain an adequate immune response before they are exposed [36].

In contrast, emergency vaccination may be followed very shortly by challenge [36]. For this reason, emergency vaccines usually have higher doses of antigen, to create immunity faster. It is believed that a highly potent vaccine is more likely to protect against a heterologous virus than an equally cross-reactive but less potent vaccine, although this effect may not occur with all strains [13;59;118;137]. Some well established vaccine strains such as A22 Iraq, O1 Manisa, O1 Lausanne, O1 Campos and A24 Cruzeiro are very potent and protect against a range of field strains within the same serotype [1]. 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 [13;137]. Both conventional and emergency vaccines
supplied by reputable manufacturers and banks usually have PD<sub>50</sub> levels well over the minimum stipulated values [1;36;59]. In a recent 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 [139]. 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 [38].

21. 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 1/3 to ½ of the cattle dose, using either oil or aluminum hydroxide adjuvanted vaccines [29].

Although needle inoculation is currently used to deliver FMD vaccines, needle-free devices are expected to be more efficient, and may maximize the number of vaccine doses that can be obtained from antigen concentrates. Needle-free devices have not been validated with FMD vaccines, and very few published studies have explored the use of these devices. In one experiment in pigs, intradermal (ID) vaccination with a 1/10 dose of FMD antigen, using a needle-free device, was promising, although optimization of the dose could not be achieved [195]. 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.

22. MATERNAL ANTIBODIES

Maternal antibodies to FMD may be detected in the offspring of vaccinated dams. In calves born to nonvaccinated cows, some vaccines can be given as early as 1 week of age [13]. 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 [39]. The OIE suggests delaying vaccination as long as possible to allow maternal antibodies to diminish, but no longer than 4 months [13]. Many calves will be able to respond to the vaccine by this time [13], and maternal antibodies do not usually persist longer than 6 months [38]. 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 [39]. Maternal antibodies may interfere less with oil adjuvanted vaccines than aluminum hydroxide adjuvanted vaccines in ruminants [13].
Young pigs develop poor immunity to FMD vaccination; they are usually protected by vaccinating the sow in endemic areas [28]. Breeding sows may be vaccinated twice a year or more frequently, during their pregnancy. Maternal antibodies interfere with vaccination before 8 weeks of age [28]. In pigs, the first FMD vaccines are usually given at 10-12 weeks and repeated in 2 weeks. Both NSP antibodies and neutralizing antibodies were detected in some piglets, when sows had been immunized with an unpurified FMD vaccine during an outbreak in Taiwan [165].

In kids born to goats immunized with commercial quadrivalent FMD vaccines, maternal antibodies persisted for up to 90 days after birth [111]. 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 [111]. In lambs, protective levels of maternal antibodies are also thought to persist for up to 4 weeks of age [196].

Most young buffalo seem to become infected when they are approximately 2-6 months old, after maternal antibodies have decreased ([197] reviewed in [43]).

23. LIMITATIONS OF VACCINATION
Optimal protection of each individual animal is not usually possible during mass vaccination [105;151]. 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.

23.1 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 ([116;135;136] reviewed in [137]). Acceptable tests to monitor the level of protection in vaccinated herds include the solid-phase competition ELISA, the liquid-phase blocking ELISA and VNT [13;38]. 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 [137]. Some sheep without titers at the time of challenge can be protected [153]. 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. 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.

24. 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.

25. 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.

### 25.1 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 [7]. Conversely, vaccination may allow the culling of some animals to be delayed, and relieve pressures on personnel and resources involved in slaughter and disposal. Finally, the resources and technologies needed for
associated activities, including individual animal identification, traceability and movement permitting, must be available in a vaccination campaign.

25.2 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;12;25;100]. Aerosol transmission is also influenced by factors such as the prevailing winds and humidity, as well as transmission over water vs. land [12;44]. 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 [100]. 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 [28]. 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.

Although very few modeling studies have been published for the U.S., models have the potential to help estimate the probable course of hypothetical outbreaks. However, they should be used with care. Some models employed during the 2001 outbreak in the U.K. did not predict the actual course of the epizootic well [12;44]. 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 [13;19]. However, the virus can infect some wildlife populations for a time [19;20;32;33], 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.

25.3 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 [198]
- Variable or recurrent costs including the cost of vaccines and delivery [198]
- Costs to identify vaccinated animals, permit their movement, and conduct serosurveillance 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 [198].

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].
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 [198]. 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 [198]. However, if culling is more widespread or the disease is out of control, vaccination may save livelihoods [198].

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 [198]
- Prevent national markets from being disrupted or rapidly restore them [198]
- Minimize other economically important factors such as the disruption of tourism or impacts on local economies [198]
- Reduce the time export markets are lost

Vaccination may be particularly beneficial to small-scale operators whose safety nets are limited [198]. 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 [198]. The emotional impact of the destruction of apparently healthy animals should also be taken into consideration [198]. 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 [121].

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 [198]. 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 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 [198]. 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. It also implies that delaying the implementation of a vaccination-to-live program decreases its economic and epidemiological benefits. 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.

25.4 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.

25.5 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 [38]. 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. 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.

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 [12].

26. 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 [20]. 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 [20]. 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 [20]. 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 preventative 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 nasomaculatus*), 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 [20].

Only killed vaccines should be used in zoo animals, unless the vaccine has been specifically tested in the target species [20]. 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 Ad5-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 [199]; however, Ad5-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 [20]. 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 ([200] reviewed in [20]). The vaccine was administered subcutaneously at the cattle dose of 3 ml, 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 [20]. 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 [20]. 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.

### 27. 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
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 [72]. Concerns about the ethics of killing large numbers of healthy animals were also reported during the 2001 outbreak in Taipei, China [190].

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 [12]. Concern that milk and meat could not be sold was another factor in rejecting vaccination-to-kill during this epizootic [12]. 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 [198].

### 27.1 Foot and Mouth Disease as a Zoonosis

FMD is not considered to be a public health threat [201]. FMDV can be zoonotic, but infections in humans are thought to be very rare [202]. Exposure to extremely large amounts of virus or a predisposing condition may be necessary for infection with this virus [203]. Between 1921 and 1969, more than 40 laboratory-confirmed cases of FMD in humans were published [202]. In three laboratories, 15% to 54% of workers were seropositive, but no clinical cases were reported [202]. 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 [202]. There are no reports of FMD infections among children who may have been exposed in contaminated smallpox vaccines [202].

Infected humans may be subclinically infected or develop vesicular lesions and influenza-like symptoms [202;203]. 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 [203]. Cases have also been reported after drinking unpasteurized milk. In 1834, three veterinarians apparently infected themselves by deliberately drinking raw milk from infected cattle for three days [202;204]. No cases of FMD have been reported after eating meat from infected animals [203]. Person-to-person transmission has never been reported [204]; however, vesicles from affected people do contain virus [202]. Reports of FMD in humans have become even more rare since vaccination reduced the incidence of this disease [202].
27.2 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 [149]. 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. [12].

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 [38].

27.3 Procedures to Inactivate FMDV in Animal Products
FMDV in meat can be inactivated[38] 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[38] 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.

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 [38]. 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 [38].

27.4 Procedures for Marketing Animal Products After Emergency Vaccination
In the UK, plans have been established for meat and milk use after emergency vaccination [100]:

- 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.

27.4.1 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 [198]. 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 [12;205]. Some supermarket chains claimed they would not sell milk from vaccinated cattle [12].

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 [205]. 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 ([206] reviewed in [205]). 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 [205].

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 [205]. 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 ([207] reviewed in [205]). 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 [205]:

- 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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Disease Center
30. ACKNOWLEDGEMENTS

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<td><strong>AGID</strong></td>
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<td>Center for Veterinary Biologics; a division of APHIS</td>
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<td>Department for Environment, Food, and Rural Affairs; division of United Kingdom government</td>
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<td><strong>DOI</strong></td>
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<td>Duration of Immunity</td>
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<td>Differentiating Infected from Vaccinated Animals</td>
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