Foot-and-mouth disease (FMD) is one of the most economically and socially devastating diseases affecting animal agriculture throughout the world. Although mortality is usually low in adult animals, millions of animals have been killed in efforts to rapidly control and eradicate FMD. The causing virus, FMD virus (FMDV), is a highly variable RNA virus occurring in seven serotypes (A, O, C, Asia 1, Sat 1, Sat 2 and Sat 3) and a large number of subtypes. FMDV is one of the most infectious agents known, affecting cloven-hoofed animals with significant variations in infectivity and virus transmission. Although inactivated FMD vaccines have been available for decades, there is little or no cross-protection across serotypes and subtypes, requiring vaccines that are matched to circulating field strains. Current inactivated vaccines require growth of virulent virus, posing a threat of escape from manufacturing sites, have limited shelf life and require re-vaccination every 4–12 months. These vaccines have aided in the eradication of FMD from Europe and the control of clinical disease in many parts of the world, albeit at a very high cost. However, FMDV persists in endemic regions impacting millions of people dependent on livestock for food and their livelihood. Usually associated with developing countries that lack the resources to control it, FMD is a global problem and the World Organization for Animal Health and the United Nations’ Food Agriculture Organization have called for its global control and eradication. One of the main limitations to FMDV eradication is the lack of vaccines designed for this purpose, vaccines that not only protect against clinical signs but that can actually prevent infection and effectively interrupt the natural transmission cycle. These vaccines should be safely and inexpensively produced, be easy to deliver, and also be capable of inducing lifelong immunity against multiple serotypes and subtypes. Furthermore, there is a need for better integrated strategies that fit the specific needs of endemic regions. Availability of these critical components will greatly enhance the chances for the global control and eradication of FMDV.

**Keywords:** DIVA test • foot-and-mouth disease control • inactivated vaccine • modified live vaccine • molecular vaccine • recombinant vector vaccine

More than 113 years ago, Friedrich Loeffler, together with his colleague Paul Frosch from the Robert Koch’s Institute of Infectious Diseases in Berlin, reported that foot-and-mouth disease (FMD) was caused by a filterable agent, and this is considered the first description of an animal disease caused by a virus [1]. The report was from the Research Commission on FMD, headed by Loeffler and Frosch, and set up the previous year by the Prussian Ministry of Culture and tasked, among other things, with developing a vaccine against the devastating disease causing “severe economic damage to the country’s agriculture” [2]. Inactivated FMD vaccines have been available since the early 1900s and have been instrumental in eradicating FMD from some parts of the world and repressing clinical disease in others. However, despite the use of billions of doses of inactivated FMD vaccines every year, the disease remains active, affecting millions of animals around the globe and causing economic hardship in some of the poorest regions. Today, FMD remains the main sanitary barrier to the commerce of animals and animal products. The characteristic clinical signs of FMD, which has high morbidity but low mortality, include high fever, anorexia, reduction in milk production and vesicular lesions that
appears in the mouth and feet of affected animals 2–4 days after infection [3,4]. Inactivated FMD vaccines are effective in preventing clinical disease but do not necessarily prevent viral replication at the primary site of infection, which can result in persistent infections in approximately 50% of naive and vaccinated animals exposed to FMD virus (FMDV) [5]. Although the role of these persistent carrier animals on the FMD transmission cycle remains unclear, its occurrence is one of the main deterrents to using vaccination in this rapid control and eradication of disease outbreaks in FMD-free countries. Another problem with inactivated vaccines is the presence of nonstructural proteins in some vaccine formulations, which interferes with our ability to serologically distinguish infected from vaccinated animals (for details see ‘differentiating infected from vaccinated animals’ section to follow).

There is a clear need for a new generation of FMD vaccines with an improved profile, capable of not only preventing clinical signs but that can also prevent infection and have the necessary antigenic markers to allow differentiating infected from vaccinated animals (DIVA) testing. These new vaccines need to be fit-for-purpose to address the inadequate short duration of immunity provided by commercial inactivated FMD vaccines, and importantly, their narrow range of antigenic coverage. This is especially important for endemic areas where several FMD viral subtypes may be circulating, complicating the logistics of implementing an effective vaccination program when resources are scarce. Other attributes include the need to accelerate the onset of immunity (currently 7 days) [6] and provide negative markers to effectively distinguish infected from vaccinated animals. In this article we will provide a broad overview of existing FMD vaccines and promising new technologies in the pipeline that might become available in the coming years, and provide vaccine characteristics currently lacking in commercial FMD vaccines. Based on this knowledge, we will discuss the feasibility of global strategies for the control and, when feasible, eradication of FMDV using existing and novel vaccines.

Inactivated FMD vaccines
Industrial production of inactivated vaccines began in the 1950s using the Frenkel method of culturing tongue epithelium collected from healthy slaughtered animals and infecting these cultures with FMDV, followed by collection of the virus-containing medium and virus inactivation using formaldehyde [7]. As one can imagine, the method of using primary cells limited large-scale production and was prone to contamination with adventitious agents. In addition, formaldehyde inactivation was often incomplete, resulting in infective vaccines. In the 1960s, cell-suspension cultures replaced tongue epithelium and later on ethylene imines replaced formaldehyde inactivation [8,9]. Current commercial FMD vaccines consist of inactivated purified antigen (killed virus) devoid of nonstructural viral proteins, usually by chromatographic purification, and formulated with various proprietary adjuvant formulations. These vaccine formulations have proven effective in reducing clinical disease in FMD-endemic areas and have been successfully used as an adjunct treatment in disease eradication programs in Africa, South America and Europe [2].

Foot-and-mouth disease vaccines traditionally represent the largest share of the veterinary vaccine market worldwide in terms of sales, with 26.4% of the entire livestock biological business [10]. Significant steps have been made to improve the quality of FMD vaccines, including changes in the manufacturing process to enable the differentiation of infected from vaccinated animals, but there are important variations between different manufacturers, and between vaccines distributed for FMD-endemic regions versus FMD-free countries. Owing to the high variability of FMDV serotypes and subtypes, the antigen composition of FMD vaccines is tailored for specific world regions and in many cases to specific countries or regions within them. The use of the vaccine in FMD-endemic regions requires an in-depth investigation of the epidemiology of disease and vaccine matching studies to determine whether the vaccine will be effective against the strain(s) circulating in the target area and to ensure the actual profile of the vaccine is suitable for control and eradication [11]. There are primarily three formulations that represent the large majority of all commercial inactivated FMD vaccines worldwide:

- High-potency vaccines (emergency use)
- Oil-emulsion conventional vaccines (routine control)
- Aluminum hydroxide vaccines (for cattle)

Emergency use (high-potency) FMD vaccines
Due to the instability of the formulated product (12–18 months), vaccines for emergency use are usually stored as frozen antigen concentrates in vaccine banks by FMD-free countries or groups of countries (for review see [19]). For example, the USA, in cooperation with Canada and Mexico, has a tripartite ‘North American Foot and Mouth Disease Vaccine Bank’. Vaccines formulated from frozen antigen banks contain at least six PD50, provide an onset of protection from challenge within 4–7 days post-vaccination (dpv; partial immunity as early as 2 dpv) in cattle, swine and sheep, and some provide wider antigenic coverage and protection for heterologous FMDV subtypes within a serotype [6,18–20]. Duration of protective immunity after single vaccination is limited, requiring re-vaccination after 6 months [11,21,22]. Vaccines decrease clinical disease, virus amplification (shed and spread) and reduce the number of persistent infections in vaccinated ruminants challenged with FMDV [18,23,24]. Bulk antigens stored in
vaccine banks require at least 3–4 days for formulation and finishing of vaccine from frozen antigen concentrates, and 2–3 additional days for shipping resulting in deployment delays. This does not take into account the time required for completion of quality control tests to demonstrate purity, safety and potency, which might be a requirement of some biological regulatory agencies.

**Conventional (ready-to-use) oil-emulsion FMD vaccines**

Oil-adjuvanted vaccines formulated with a potency of at least 3 PD₅₀ have been shown to provide an onset of protective immunity within 7 days in cattle, swine and sheep [6,19,25]. Vaccines decrease clinical disease, and virus amplification (shed and spread), but do not prevent the establishment of persistent infections in up to 50% of ruminants vaccinated and later challenged with wild-type FMDV [5,6,24]. Differences in efficacy and potency have been reported between double oil-emulsion versus water-in-oil single-emulsion formulations with a higher antibody response reported for double oil-emulsion [12,13,26]. Enhancement of the immune response induced by the inclusion of saponin in oil-adjuvanted vaccines has been reported [27]. Oil-adjuvanted vaccines are the most used worldwide but, in order to maintain sufficient levels of immunity to suppress occurrence of clinical disease, revaccination must be carried out every 6 months [15]. After multiple doses of vaccines in older animals, vaccination frequency could be decreased to once a year, provided that no new strains not covered by the vaccine formulation emerge or are introduced [28–31].

One of the shortcomings of the current conventional vaccine is the short shelf life once it is formulated either as double-oil emulsion or aluminum gel (for details see [32]).

**Aluminum hydroxide–adjuvanted vaccines**

The aluminum hydroxide–saponin formulation originally developed for FMD vaccines has some advantages such as ease of production and antigen concentration by adsorption to aluminum hydroxide gels [32]. However, aluminum hydroxide-adjuvanted vaccines have several disadvantages over oil-emulsion vaccines, including the fact that they cause granulomas at the inoculation site [33] and are not as effective in swine as oil-emulsion vaccines [34,35], have a shorter shelf life than oil-emulsion vaccines, are less potent per microgram of antigen, and produce a shorter duration of immunity [32]. However, these vaccines continue to be produced around the world for use mostly in ruminants.

**New experimental vaccine platforms**

Since structural proteins are the main antigens responsible for inducing protective responses [36,37], several attempts have been made to improve current inactivated FMD vaccines by utilizing cloned capsid proteins expressed by rDNA technology. However, the subunit vaccines produced in *Escherichia coli* and peptide vaccines induce narrow immune responses that the virus easily gets around through antigenic drift mechanisms [38]. Recently, significant improvements in rDNA-based vaccines have been made offering improvements in efficacy, safety and use in disease control and eradication [39]. Most of these improvements consist of introducing mechanisms for protease processing of the viral capsid proteins that result in structurally more complex antigens. Some of these new approaches are described in the following sections [40].

**DNA vaccines**

Vaccination using plasmid DNA containing FMDV sequences has been reported as an efficient way to induce protective immunity in the mouse model [41,42]. However, protection by DNA vaccination in farm animals such as cattle, sheep and pigs has proven more challenging and requires multiple doses and addition of adjuvants and cytokines (e.g., GM-CSF, IL-2) to induce only partial or in some cases full protection [42,43]. In most DNA vaccines the antibody response, which is critical in protection against FMDV, is both limited and short lived [44]. Despite these shortcomings, DNA vaccines are appealing because plasmid DNA does not require high containment facilities for manufacture, is relatively stable for storage, allows for the rapid incorporation of emerging field strain sequences and allows discrimination between infected and vaccinated animals [41,48]. Another interesting feature of DNA vaccines is that they have been reported to induce protection not only against clinical disease but also prevention of the carrier state in sheep [43]. Other applications of DNA vaccines include a prime–boost approach using inactivated vaccines to improve their efficacy [45]. Using priming with a plasmid DNA-containing capsid and some of the nonstructural proteins of FMDV followed by boost with inactivated vaccine and recombinant 3D protein resulted in high antibody titers and protection of swine not only against homologous but also against heterologous challenge. Although not a practical approach at this time, this promising research should continue.

**FMD peptides**

In laboratory animal models (e.g., mice, guinea pig), several FMD capsid-based peptide vaccine candidates have been shown to induce peptide-specific anti-FMDV serum-neutralizing (SN) antibody titers, and in some instances have been shown to confer protection against FMDV challenge [46,47]. Unfortunately, these positive results in laboratory animal models have not been consistently reproduced in cattle and pigs [25]. Although early studies in cattle showed promise [48], in a large-scale synthetic-peptide vaccination study in 138 cattle using four different FMDV serotype C VP1 G-H loop-based peptides, none of the peptides, tested at several doses and vaccination schedules, conferred protection in above 40% of the vaccinated animals [38]. Notably, several mutant FMDV strains were isolated from vaccinated cattle, suggesting that peptide vaccination induced the rapid generation and selection of FMDV antigenic variants in *vivo*.

Efforts to improve and broaden VP1 G-H loop peptide immunogenicity through the incorporation of T helper (Th) sites and consensus residues into the hypervariable positions (UBI peptide) resulted in a high level of protection in swine following FMDV 01 Taiwan challenge [49]. A subsequent pilot study in cattle showed that the UBI peptide induced peptide-specific antibodies but relatively low SN titers, and failed to protect cattle following FMDV type O challenge at 3 weeks post-vaccination [25].
However, a peptide vaccine is now commercially available [202]. FMDV peptide vaccine adjuvanted with cholera toxin and administered transcutaneously elicited antipeptide antibodies with enhanced virus neutralizing activity in mice [50]. However, further experiments demonstrating efficacy in target species are still required. Recent studies in swine utilizing nontoxic \textit{Pseudomonas aeruginosa} exotoxin A expressing the FMDV VP1 G-H loop failed to induce protective immune responses [51].

The recent development of dendrimeric peptides containing one copy of an FMDV T-cell epitope branching out into four copies of a B-cell epitope provides potential improvements over the conventional linear peptide [52]. Pigs vaccinated with a dendrimeric peptide and subsequently challenged with FMDV did not develop significant clinical signs, appear to have abrogated systemic and mucosal FMDV replication, and did not transmit the virus to contact controls. The dendrimeric peptide used in this experiment elicited an immune response comparable to that found for control of FMDV-infected pigs. Dendrimeric designs for other FMDV serotypes and subtypes need to be developed and tested, but this new technology provides substantial promise for peptide-subunit vaccine development.

**Virus-like particles**

Foot-and-mouth disease virus-like particles (VLPs) are nonreplicating, nonpathogenic particles that have structural characteristics and antigenicity similar to the parental virus. They are similar in conformation to intact virions and are formed by the self-assembly of processed capsid proteins. A critical component of VLP experimental vaccines is the ability to process the viral capsid polyprotein (P1) into cleaved products that can then assemble into VLPs. There are different protein-processing paths that have been pursued by the inclusion of nonstructural viral proteins 2A, 2B and 2C. There are several expression systems for the production of VLPs, including:

- Various mammalian cell lines, either transiently or stably transfected or transduced with viral expression vectors [55,56]
- The baculovirus/insect cell and larvae systems [57–59]
- Various species of yeast including \textit{Saccharomyces cerevisiae} and \textit{Pichia pastorii} [60]
- \textit{E. coli} and other bacteria [61–63]

A yeast-derived VLP experimental FMD vaccine was initially described in 2003 [60]. The capsid from a serotype O strain induced SN and ELISA titers in guinea pigs and these animals were protected against homologous challenge. More recently, co-expression of either recombinant bovine IFN-\gamma [64], IL-18 [65] or HSV-70 [66] and VP1 [64,65,67] constructs has been shown to enhance SN and cell-mediated immune responses in mice; however, no livestock vaccine efficacy studies have been reported.

Baculovirus-derived VLP experimental FMD vaccines have been shown to provide some protection against clinical disease in swine, but fail to protect against viral replication. Similar results using an \textit{E. coli}-derived VLP experimental vaccine were also reported [37]. Recent reports have shown improvements by using baculovirus and silkworm larvae to express FMDV P1 and including protein 2A at strategic sites to facilitate processing of VP1–VP2 and VP0 [99]. The authors provide electron microscopy evidence of VLP assembled in the larvae lysates. Furthermore, vaccines prepared in this fashion for serotypes Asia and O conferred protection when used to immunize cattle [57]. Further testing is necessary to determine the feasibility of this approach for large-scale production of FMD vaccines for livestock.

Hepatitis B virus core (HBc) particles self-assemble into capsid particles and are extremely immunogenic. However, formation of VLPs can be restricted by size and structure of heterologous antigens. The first report of the use of the HBc system for expression of amino acids 141–160 of the VP1 protein of FMDV was made over 20 years ago, and the immunogenicity of the VLP structures was reportedly similar to that of intact FMD particles [68]. Very recently, the formation of VLP in mammalian cells by modified HBc fused with specified FMDV multi-epitopes was studied. Complete VLP structures with one construct was confirmed by electron microscopy and induced both humoral (peptide- and FMDV-specific antibody) and cell-mediated immunity responses in mice [69].

The generation of VLP experimental FMD vaccines using transgenic plants has also shown some laboratory success. \textit{Arabidopsis thaliana}-transformed plant extracts expressing the FMDV VP1 gene were shown to provide protection against FMDV challenge in mice [70]. Similar studies have also been reported using transgenic potato plants [71] or alfalfa plants [72] as immunogens in serology and challenge studies in mice. Related studies using HBc to express a VP1 capsid epitope in transgenic tobacco has also been reported [73]. To date, none of these transgenic plant-derived VLP experimental vaccine candidates have been tested for efficacy and safety in cattle or swine, and the regulatory and manufacturing path for transgenic plant-derived vaccines is not well defined.

**Viral-vectored FMD vaccine platforms**

Viral vectors have been successfully used to deliver sequences coding for FMDV capsid proteins into animals. There are several examples including herpesvirus (pseudorabies), poxviruses and \textit{a}-virus vectors. However, the best-documented and most effective platform uses human defective adenovirus 5 (hAd5). Unlike experimental DNA vaccines that mainly target the expression of the specific antigens to be presented to the immune system on the surface of transfected cells, the purpose of this platform is to provide the genetic information to express and process all the FMDV structural proteins, presumably resulting in the formation of virus-like particles in the hAd5-infected cells; although direct evidence of this has not been clearly shown [74]. The hAd5–FMD vaccines are the most extensively tested and were shown to be as effective as inactivated vaccines against FMD. Complete protection has been shown both in swine and cattle receiving one vaccine dose and challenged as early as 7 dpv [75–77]. The hAd5–FMD vaccine platform contains all FMDV capsid proteins and nonstructural proteins 2A and 3C, but lacks other nonstructural proteins such as 3B and 3D. These two proteins are the targets of serological tests aimed at differentiating infected from vaccinated animals, making the hAd5–FMD vaccine an attractive candidate for livestock vaccination programs.
were too attenuated and did not consistently induce protection. Attenuating mutations or gene deletions were better characterized but batches and tested in cattle in the US mainland (for the first time). Serum from animals inoculated with this leaderless marker lysing leader-deleted FMDV achieved a single immunization and intra-dermal lingual (tongue) or contact protection against generalized FMD disease and viremia following the most advanced product candidate in development. To date, this vaccine has been tested in over 150 cattle and shown to provide protection against generalized FMD disease and viremia following a single immunization and intra-dermal lingual (tongue) or contact challenge at 7 or 21 dpv [77]. Similarly to the commercially inactivated vaccines, protection as early as 4 dpv has been demonstrated in some, but not all animals [76]. These new molecular FMD vaccine candidates are currently being manufactured in experimental batches and tested in cattle in the US mainland (for the first time in US history) as part of the veterinary licensing process [77].

**cDNA-derived inactivated FMDV vaccine platform**

The utilization of cDNA-derived FMDV as a safe candidate platform for production of inactivated vaccines was first reported utilizing leader-deleted FMDV [78–80]. Attenuation of FMDV was achieved by manipulating the genome to eliminate the Lpro coding sequence, which is known to be involved in FMDV pathogenesis in vivo. In a pilot study, this virus platform was shown to be completely attenuated in cattle based on absence of tongue lesions following direct inoculation of susceptible cattle [79]. Once inactivated, the antigenic properties of the cDNA-derived FMDV vaccine are expected to be those obtained from inactivated wild-type virus. More recently, this platform has been improved by adding unique restriction enzyme sites for rapid swapping of capsid sequences and also by deleting specific epitopes in nonstructural proteins (NSPs) 3B and 3D allowing for serological DIVA testing [Rieder E. Pers. Comm.]. Serum from animals inoculated with this leaderless marker virus can be readily distinguished from parental FMDV-infected animals utilizing DIVA serological tests such as competitive ELISA for NSP. Accordingly, unlike conventional inactivated FMD vaccines, this platform eliminates the need to remove NSP during the manufacturing process, and since the vaccine virus is attenuated, eliminates the concern associated with the manufacture of live FMDV if the vaccine virus escapes from the manufacturing facility.

**Modified live vaccines**

In the past few years, development of live-attenuated FMD vaccines has shown limited success owing to unstable phenotype, differences in attenuation among various livestock species (e.g., attenuated in cattle but not in swine) or vaccines that were incapable of consistently inducing protective immune responses [81–85]. Some of these vaccines relied on the use of viruses selected in cell culture or in laboratory animals showing attenuated phenotypes, but the mechanisms of attenuation were largely unknown and attenuation was often incomplete and reversible [81]. Other vaccines engineered to contain attenuating mutations or gene deletions were better characterized but were too attenuated and did not consistently induce protection [85]. Owing to these problems and concerns over reversion to virulence through mutation or recombination with field viruses, live FMD vaccines have not been developed. However, new technologies such as the development of reverse-genetic cDNA systems for FMDV provides new opportunities for identifying virulence determinants in the FMDV genome. As new virulence determinants are identified, new possibilities for attenuated vaccines will arise. Translating this knowledge into vaccine candidates will require detailed understanding of the virus–host interaction and mechanisms of pathogenesis in order to address concerns about complete attenuation in all susceptible species, and even eliminating the possibility of reversion to virulence. All this is now possible given the current availability of well-established infectious cDNA FMDV systems and the ability to engineer multiple specific mutations in critical elements that regulate the virulence of the virus [86–90]. Importantly, this methodology also allows the engineering of negative antigenic markers to include DIVA capability.

**Adjuvants & biotherapeutics**

The FMDV incubation period can be as short as 2 days and animals can shed virus prior to signs of generalized disease [3,4]. Since FMD vaccines generally require at least 4–7 days for protective adaptive immunity to develop, it is critical that FMD control programs address the gap in the onset of immunity provided by current vaccines to limit and control disease spread. With recent breakthroughs in our understanding of antiviral innate defense mechanisms, biotherapeutics or immunomodulators offer the potential for their use as an emergency tool to stop viral shed and spread within 24 h after administration [91–93]. When used in combination with rapid-acting vaccines, it may now be possible to elicit early short-term anti-FMDV effects until the onset of vaccine-induced protective immunity.

It has been previously shown that FMDV replication is inhibited by type I interferons (IFN-α/β) [94,95]. At least two IFN-α/β-stimulated genes, double-stranded-RNA-dependent protein kinase and 2',5'-oligoadenylate synthetase/RNase L, are involved in this process [95]. It has been demonstrated that an Ad5 vector containing the porcine IFN (pIFN-α) gene (Ad5–pIFN-α) induces high levels of biologically active interferon when injected in swine. Furthermore, swine inoculated with a single dose of Ad5–pIFN-α were completely protected when challenged with FMDV 1 day later [94]. The level of protection correlated with the Ad5–pIFN-α dose and the level of plasma IFN-α. Additional studies demonstrated that Ad5–pIFN-α treatment alone can protect swine from challenge for 3–5 days and can reduce viremia, virus shedding and disease severity when administered 1 day post challenge. Importantly, a combination of Ad5–pIFN-α and Ad5–FMDV vaccination provided both immediate and long-term protection in swine [96,97]. Type II pIFN (pIFN-γ) also has antiviral activity against FMDV and, in combination with pIFN-α, has a synergistic antiviral effect [92]. The results indicate that the combination of type I and II interferons act synergistically to inhibit FMDV replication in vivo and confer protection against challenge. Similar studies in cattle have only shown partial but not complete protection against FMDV challenge [91].
Differentiating infected from vaccinated animals

Exposure to structural FMDV capsid proteins as well as NSPs such as a RNA polymerase through infection or vaccination will induce the production of antibodies to these proteins. The removal of NSPs from FMD vaccines enables the differential detection of antibodies to the NSPs in FMDV-infected animals. Thus, current DIVA strategies for FMD are based on the use of a diagnostic test that can differentiate the detection of antibodies to NSPs in infected versus vaccinated animals. For detailed information on DIVA tests and their role in control and eradication please see [98–100].

Although the application of current DIVA strategies has been successfully implemented on a herd basis, there is still some concern of vaccinated animals becoming asymptomatic virus carriers without positive reaction to NSP serological tests [101]. Of particular concern is the fact that current vaccines may have residual NSPs (depending on the manufacturing process) that could result in the detection of NSP antibodies in vaccinated animals if multiple doses of vaccine are applied annually [102–104]. In addition, the application of the current DIVA strategy is dependent on diagnostic tests originally developed to determine FMDV infection and used primarily for surveillance and not necessarily as companion tests to vaccines [100,105].

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

**Expert commentary**

Global control and eradication of FMD has been proposed for many years. Recently in the Paraguay declaration of 2009, the World Organization for Animal Health, the Food Agriculture Organization and other international agencies and numerous governments set the goal for global eradication of FMD by the year 2030 [201]. Although such declarations are important and eradication seems within reach, particularly in regions where FMD is largely controlled by vaccination, in other regions, where FMD is rampant and FMD control competes with other basic needs such as food, human health and education, such an eradication goal seems unlikely. There is a clear need for alternative control methods, particularly vaccines that can address the major shortcomings of current and even upcoming vaccines. These fit-for-purpose vaccines accompanied by appropriate diagnostics and control strategies should address the needs of each region and particularly induce long-term immunity sufficient to break the endemic transmission cycles that currently maintain the virus circulating in these areas [106]. Since funding for such vaccines, diagnostics and control strategies is limited worldwide, it is important to leverage international capabilities into aligned efforts toward common achievable goals. Such an effort requires international alliances such as the Global Foot and Mouth Disease Research Alliance [203], launched in 2003 as a worldwide association of animal research organizations, that are involved in combating FMD. Its aim is to build a global alliance of partners to generate and share knowledge – in a virtual FMD laboratory – to develop tools that can better combat the threat of the disease. Collaborative research is central to the fulfillment of FMD global eradication.

**Five-year view**

To achieve the goal of a global effort for FMD control and eradication, the next 5 years are critical. Large parts of the world such as South America are at the brink of eradicating FMD with currently available tools, while others will require new tools.
and sustainable approaches that are relevant to their economy and methods of livestock production to achieve the goal of eradication. Without continued effort being placed in the necessary research for the development of better control strategies and appropriate tools, the goal of eradication will unlikely to be realized. The focus of the research should be centered on goals that will address the major problems limiting control and eradication, including understanding of the ecology, pathogenesis and transmission of FMDV in relevant species, and importantly, the development of new and cost-effective technologies designed to provide broad-range and long-lasting protective immunity after vaccination. The information derived from this research effort should lead to vaccines that have the necessary characteristics (listed in Table 1) that will allow the global control and eventual eradication of FMD.

Acknowledgements
The authors would like to thank members of the Global Foot-and-Mouth Research Alliance (GFRA) and the US National Veterinary Stockpile, Foot-and-Mouth Countermeasures Working Group for their assessment of foot-and-mouth countermeasures.

Financial & competing interests disclosure
The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

No writing assistance was utilized in the production of this manuscript.

Key issues
• Foot-and-mouth disease is a devastating disease of farm animals that negatively affects the livelihoods of millions of people around the world.
• Control programs based on test-and-slaughter policies without vaccination are not feasible in endemic countries, most of which are developing countries highly dependent on livestock for their economy and the health of their citizens.
• Success in eradicating the disease in Europe and in most of South America was achieved after sustained and expensive mass vaccination efforts, which are required to attain herd immunity.
• Global eradication using current mass vaccination strategies may not be feasible for impoverished regions that need it the most and can afford it the least.
• Existing foot-and-mouth vaccines are not suitable for global eradication because they:
  – Induce short-term protection requiring re-vaccination every 6 months or less
  – Do not cross-protect against the multiple viral serotypes (seven) and subtypes (dozens)
  – Have a short shelf life and are heat labile requiring cold chain from production to delivery
  – Require expensive biosafety level 3 facilities for growing live virus during vaccine production
• There is a need for vaccines capable of preventing infection and inducing life-long immunity against multiple serotypes and subtypes.
• These vaccines should be safely and inexpensively produced, and easy to deliver.
• There is a need for better integrated strategies that fit the specific needs of endemic regions.
• Only when these critical components are available will the global eradication of foot-and-mouth disease virus be possible.

References
Papers of special note have been highlighted as:
• of interest
** of considerable interest
• Provides a valuable historical perspective to foot-and-mouth disease (FMD) research from the description of the viral serotypes and subtypes to the research leading to current vaccine technology.
• The carrier state in ruminants is one of the major issues in FMD control and eradication. This manuscript provides a good overview of FMD persistence in ruminants and the resulting carrier state.
•• The fascinating history of FMD vaccines and vaccination program illustrates the fact that we still face many of the same problems with FMD that were faced 50 years ago during eradication efforts in Europe.

- The development of new-generation FMD vaccines will face the same challenges as vaccines to other high-consequence pathogens; with a limited market and the prospect of eradication, private investment is limited.


Doel provides a state-of-the-art description of the FMD vaccine manufacturing process. This comprehensive review touches on every step of the vaccine manufacturing and their associated challenges.


Review

Development of vaccines toward the control & eradication of foot-and-mouth disease


Few studies have explored development of live-attenuated FMD vaccines. This example describes efforts that resulted in an over-attenuated virus that did not induce sufficient protection.


Websites


202 United Biomedical Products www.unitedbiomedical.com/Product

203 Vision of GFRA www.ars.usda.gov/GFRA

Development of vaccines toward the control & eradication of foot-and-mouth disease