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Clinical Diagnosis, Epidemiological Patterns, and Prophylactic Strategies for Foot-and-Mouth Disease in Ruminants and Swine

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ABSTRACT

Foot-and-mouth disease (FMD) is a highly contagious transboundary viral disease of cloven-hoofed animals, including cattle, buffalo, sheep, goats, and swine, and remains a major constraint to livestock productivity and international trade. The causative agent, foot-and-mouth disease virus (FMDV), is a positive-sense single-stranded RNA virus of the genus Aphthovirus (family Picornaviridae) with seven immunologically distinct serotypes (O, A, C, Asia1, SAT1, SAT2, SAT3), and limited cross-protection between serotypes complicates both vaccine selection and outbreak containment. Disease transmission occurs through direct contact, contaminated fomites, animal products, and airborne spread, and outbreaks are amplified by animal movement networks, market systems, and wildlife–livestock interfaces. Clinically, FMD is characterized by fever, salivation, lameness, and vesicular lesions of the oral mucosa and feet, but definitive differentiation from other vesicular diseases requires laboratory confirmation. This review synthesizes recent advances (2020–2025) in FMD epidemiology, diagnostic approaches, and prophylactic strategies in ruminants and swine. Diagnostic methods are discussed across clinical assessment, serology (VNT, ELISA formats including DIVA-compatible assays), virus isolation, and molecular platforms such as RT-PCR, real-time PCR, RT-LAMP, multiplex PCR, microarray-based detection, and point-of-care CRISPR/Cas systems. Preventive and control measures are critically evaluated, including movement restriction, biosecurity, stamping-out strategies, vaccination programs (inactivated, polyvalent, marker vaccines, vector-based and oral candidates), and emerging technologies such as nanoparticle-based adjuvants, enhanced antigen-stability platforms, and genomic surveillance via next-generation sequencing. Despite substantial progress, persistent challenges include serotype and lineage evolution, vaccine matching gaps, carrier-state uncertainties, and limited access to high-quality diagnostics in endemic settings. Strengthening integrated surveillance, expanding field-deployable diagnostics, and accelerating development of broadly protective and DIVA-compatible vaccines are essential for sustainable control and eventual elimination of FMD.

Keywords
Foot-and-Mouth Disease; Foot-and-Mouth Disease Virus; Ruminants; Swine; Epidemiology; Transmission; Clinical Diagnosis

INTRODUCTION

Foot-and-mouth disease (FMD) is a highly contagious transboundary viral disease of major economic and veterinary importance, primarily affecting cloven-hoofed livestock including cattle, buffalo, sheep, goats, and swine (1,2). The causative agent, foot-and-mouth disease virus (FMDV), belongs to the genus *Aphthovirus* within the family *Picornaviridae* and is characterized by a single-stranded positive-sense RNA genome of approximately 8.4 kb enclosed in a non-enveloped icosahedral capsid (3,4). The viral genome encodes four structural proteins (VP1–VP4), among which VP1, VP2, and VP3 are exposed externally and represent key antigenic determinants that influence serotype specificity and vaccine performance (5,6). A defining challenge in FMD control is the existence of seven immunologically distinct serotypes—O, A, C, Asia 1, SAT 1, SAT 2, and SAT 3—along with extensive intra-serotype genetic and antigenic variation, which collectively limit cross-protection and complicate vaccine strain selection (7–9).

Clinically, FMD is associated with fever, salivation, vesicular lesions in the oral cavity and feet, lameness, and reduced productivity, with particularly severe outcomes in young ruminants where myocarditis may lead to sudden death (10–12). Even in recovered adult animals, prolonged reductions in milk yield, weight gain, and reproductive performance can persist, and the possibility of a carrier state remains an epidemiological concern in control programs (2,13). The disease imposes substantial economic losses through decreased animal productivity, high costs of outbreak control, and restrictions on domestic and international trade in livestock and animal products (14–16). These impacts are amplified in market-oriented and smallholder production systems where animal movement and mixed-species husbandry facilitate transmission and sustain endemicity (17,18).

Globally, FMD remains endemic across large parts of Asia, Africa, and the Middle East, where livestock systems are highly diverse and surveillance capacity is variable (19,20). In contrast, many regions such as Western Europe, North America, and Oceania have maintained prolonged FMD-

free status, but continue to face ongoing risk due to transboundary spread, informal trade, and the potential introduction of novel lineages through animal movement and contaminated fomites (21,22). FMDV is environmentally stable under favorable conditions and may persist in contaminated soil, feed, hair, and equipment, enabling indirect transmission over extended periods (23). The combination of high infectivity, multiple serotypes with limited cross-immunity, rapid viral evolution, and complex animal movement networks makes FMD control particularly challenging and necessitates integrated strategies that combine rapid diagnosis, targeted vaccination, biosecurity, and coordinated surveillance (8,24).

Rationale for this review

Effective management of FMD depends heavily on early and precise diagnosis, accurate serotyping, and timely implementation of control measures that are appropriate to local epidemiological conditions (25,26). However, clinical diagnosis alone is insufficient because FMD cannot be reliably distinguished from other vesicular diseases such as vesicular stomatitis, swine vesicular disease, vesicular exanthema, and Seneca valley virus infection, particularly during early outbreak phases or in atypical presentations (27,28). Laboratory confirmation therefore remains essential, yet diagnostic capacity varies widely between regions, and traditional approaches such as virus isolation and serotype-specific serology can be labor-intensive, time-consuming, and dependent on specialized containment facilities (29,30). Although antigen detection ELISA, RT-PCR, and real-time RT-PCR have become widely adopted, diagnostic performance may be influenced by sample quality, viral load, serotype bias, and the emergence of divergent lineages (31,32). This creates an ongoing need for sensitive, rapid, and affordable diagnostics that can be deployed at the point of care without compromising specificity or the ability to support surveillance and vaccine matching (33,34).

In parallel, vaccine-based control remains a cornerstone strategy in endemic regions, yet its effectiveness depends on close antigenic matching between vaccine strains and circulating field viruses, along with appropriate vaccination coverage and boosting schedules (35,36). The short duration of immunity, incomplete protection against heterologous variants, and the limited ability of conventional inactivated vaccines to prevent infection and carrier status continue to restrict long-term control, especially in settings where repeated vaccination is logistically and economically difficult (2,37). These limitations have intensified interest in improved vaccine technologies and supportive tools, including DIVA (differentiating infected from vaccinated animals) strategies using non-structural protein (NSP) markers, peptide-based and virus-like particle (VLP) platforms, recombinant and vector-based vaccines, and novel adjuvants such as nanoparticle-based systems aimed at enhancing both humoral and cellular immunity (38–41).

Furthermore, advances in genomic sequencing and next-generation sequencing (NGS) have strengthened molecular epidemiology by enabling rapid lineage tracking and supporting evidence-based vaccine strain selection (16,42). At the same time, emerging diagnostic platforms such as reverse transcription loop-mediated isothermal amplification (RT-LAMP), biosensor-based assays, and CRISPR/Cas systems offer the potential for rapid field detection and improved outbreak responsiveness, particularly in resource-limited environments (43,44). Given these developments, an updated synthesis integrating epidemiological patterns, clinical and laboratory diagnosis, and current prophylactic strategies is needed to guide veterinary public health planning and to highlight priority gaps for research and implementation.

Aim and scope (explicit)

This narrative review aims to provide an updated synthesis of foot-and-mouth disease in ruminants and swine, with emphasis on clinical diagnosis, epidemiological patterns, and prophylactic strategies for prevention and control. Specifically, the objectives are to:

summarize key epidemiological features of FMD, including distribution, transmission dynamics, and major drivers of outbreaks;

describe and critically compare clinical and laboratory diagnostic approaches, including serological, antigen-based, and nucleic acid-based methods used for confirmation and serotyping; evaluate prevention and control strategies in endemic and FMD-free settings, including movement control, biosecurity, stamping-out policies, and vaccination-based approaches; and highlight emerging technologies (e.g., genomic surveillance, NGS-based monitoring, CRISPR-assisted diagnostics, and advanced vaccine platforms including DIVA strategies and nano-adjuvants) that may strengthen future FMD management. The scope of this review focuses on FMDV infection in major domesticated livestock species—cattle, buffaloes, sheep, goats, and swine—while also acknowledging the epidemiological relevance of wildlife reservoirs and livestock–wildlife interfaces where applicable (19,45).

Review approach (very short paragraph)

A narrative literature review was conducted using electronic databases including PubMed, Google Scholar, and Scopus to identify peer-reviewed studies and reviews relevant to FMD epidemiology, clinical features, diagnostic methods, and prevention/control strategies. The primary search period emphasized publications from 2020 to 2025, supplemented by selected landmark studies to support foundational virological and control concepts. Keywords and combinations included “foot-and-mouth disease virus,” “FMDV serotypes,” “molecular epidemiology,” “RT-PCR,” “qPCR,” “ELISA,” “RT-LAMP,” “CRISPR,” “DIVA,” “vaccination,” “biosecurity,” and “outbreak control.” Articles were prioritized if they addressed ruminants and/or swine, reported outbreak investigations, evaluated diagnostic performance, described vaccine platforms or strain-matching approaches, or contributed to surveillance and policy-relevant control strategies.

VIROLOGY AND PATHOGENESIS

Foot-and-mouth disease virus (FMDV) is a small, non-enveloped virus with an icosahedral capsid belonging to the genus *Aphthovirus* in the family *Picornaviridae* (1,2). The virion encloses a single-stranded, positive-sense RNA genome of approximately 8,400 nucleotides, which is translated as a single polyprotein and subsequently processed into structural and non-structural proteins essential for viral replication and host interaction (3,4). Structurally, the capsid is composed of four viral proteins (VP1, VP2, VP3, and VP4). Among these, VP1–VP3 are surface exposed and constitute dominant antigenic sites that drive neutralizing antibody responses and determine serotype specificity, whereas VP4 is located intracapsidically and contributes to capsid stability (5,6). The high antigenic variability of surface-exposed epitopes—particularly within VP1—underpins frequent immune escape, necessitating continual monitoring of circulating strains for vaccine matching (7,8).

Seven immunologically distinct serotypes are recognized: O, A, C, Asia 1, SAT 1, SAT 2, and SAT 3 (9,10). Importantly, there is limited to no cross-protection between serotypes, and substantial genetic and antigenic diversity exists even within the same serotype due to viral evolution,

recombination, and regional lineage emergence (11,12). This diversity is a central reason why FMD remains difficult to eradicate in endemic regions and why vaccine performance may vary considerably depending on antigenic match and population immunity (13,14). Contemporary molecular studies also demonstrate that interserotypic recombination can occur, particularly in persistently infected or superinfected hosts, adding further complexity to lineage tracking and long-term vaccine design (15,16).

Host susceptibility and clinical outcomes

FMDV primarily infects cloven-hoofed domesticated livestock—cattle, buffaloes, sheep, goats, and pigs—but can also involve wild ungulates, creating opportunities for maintenance and spillover at livestock–wildlife interfaces (72,91). Susceptibility and clinical severity vary by host species, age, immune status, and viral strain, influencing both outbreak dynamics and economic burden (17,18). In cattle and swine, infection frequently begins in the oro-pharyngeal region and rapidly disseminates systemically. Typical clinical manifestations include pyrexia, depression, excessive salivation, vesicular lesions in the oral cavity and feet, erosions, and lameness, which collectively reduce feed intake, growth, and productivity (32,57,77). These clinical signs are often accompanied by substantial losses in milk yield and weight gain and may predispose animals to secondary infections due to mucosal and skin disruption (36,77).

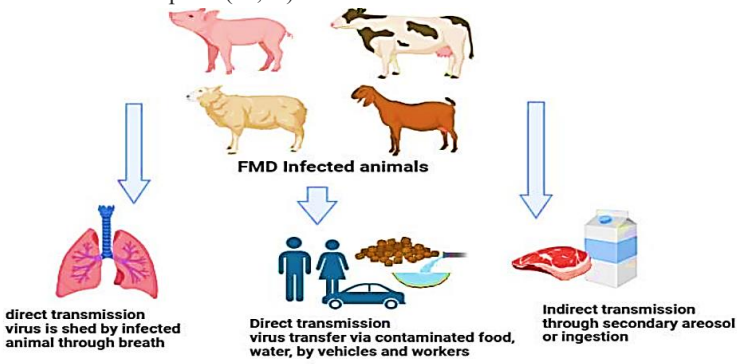

Figure 1. Transmission of foot and mouth virus

A particularly severe clinical outcome is acute myocarditis in young ruminants, which may result in sudden death with minimal or absent vesicular lesions. This "tiger heart" phenomenon represents one of the key mortality pathways in calves and is strongly age-dependent (26,104). In endemic settings, mortality in adults is generally low, but morbidity is high, and the long-term effects of productivity loss, reduced fertility, and restrictions on animal movement and trade contribute disproportionately to overall economic impact (14,36,60). Persistent infection (carrier state) in ruminants remains an ongoing concern and continues to influence control policy discussions, particularly in vaccinate-to-live strategies in FMD-free regions (84,95). At the molecular level, FMDV has evolved multiple mechanisms to antagonize innate immune responses and enhance replication. Recent studies indicate that viral structural proteins and non-structural proteins interfere with interferon pathways and host antiviral mediators, supporting immune evasion and viral persistence in infected hosts (61,100). These host–pathogen interactions are increasingly relevant for designing improved vaccines, DIVA strategies, and antiviral adjuncts, but their practical translation into field control remains an active research area (63,66).

Transmission routes and environmental persistence

FMDV transmission occurs through both direct and indirect pathways, reflecting the virus's high infectivity and ability to spread rapidly within and between herds (4,23). Direct transmission occurs via contact with infected animals, including exposure to saliva, vesicular fluid, milk, semen, and respiratory secretions. Indirect transmission is facilitated through contaminated fomites such as feed, bedding, clothing, vehicles, animal handling equipment, and farm infrastructure (23,99). Airborne spread is particularly significant for certain host species and settings, with aerosols enabling dissemination over short and occasionally long distances under favorable climatic conditions (16,73). Viral shedding may occur before obvious vesicular lesions develop, further complicating outbreak detection and early containment (2,31).

Environmental persistence of FMDV is influenced by temperature, humidity, pH, and organic matter. The virus may remain viable for extended periods in cool and moist environments, enabling continued transmission through contaminated soil, hair, feed, and equipment. Seasonal patterns of outbreaks have therefore been associated with cooler and wetter periods in several endemic regions (23,73). Epidemiologically, animal movement remains a dominant driver of spread, including local trade, livestock markets, transboundary transport, and informal movement across porous borders (4,17,40). Network-based analyses and outbreak investigations consistently highlight that high-connectivity movement systems amplify transmission risk and increase the probability of multi-focal outbreaks (67,40). Wildlife reservoirs, particularly in regions where domestic livestock co-graze with wild ungulates, may contribute to virus maintenance and periodic re-introduction into livestock populations (72,91). The combined effects of multiple serotypes, variable host susceptibility, high transmissibility, environmental persistence, and movement-driven spread explain why FMD is challenging to control and why prevention strategies must integrate surveillance, rapid diagnostics, vaccination programs, and movement/biosecurity controls in a coordinated manner (14,35,60).

EPIDEMIOLOGY AND DISEASE DYNAMICS

Global distribution and endemic versus FMD-free regions

Foot-and-mouth disease (FMD) remains one of the most economically significant transboundary animal diseases worldwide, with sustained endemicity across large parts of Asia, Africa, and the Middle East, where livestock production systems are diverse and animal movement is frequent (9,19,73). In these endemic regions, repeated outbreaks are driven by the circulation of multiple serotypes—most commonly O and A in many Asian settings, and O, A, and SAT lineages in parts of Africa—along with extensive intra-serotype genetic variation that limits cross-protection

and complicates vaccine strain selection (35,38,70). In contrast, Western Europe, North America, and Oceania have maintained prolonged FMD-free status through strict import regulations, surveillance, and rapid outbreak response capacity, but continue to face persistent risk from accidental introduction via live animal movement, contaminated animal products, and fomites (18,60).

Although some countries have achieved strong control through compulsory vaccination and effective veterinary governance, regional re-emergence continues to occur, reflecting the ongoing circulation of diverse viral lineages and the role of transboundary animal movement networks (20,27). Molecular epidemiology has increasingly demonstrated that the emergence and extinction of FMDV lineages are shaped by ecological drivers, host connectivity, and viral evolution, highlighting the need for sustained surveillance even in regions with strong control frameworks (27,70).

Drivers of outbreaks and transmission dynamics

Epidemiologically, FMD outbreaks are strongly associated with animal movement and mixing, particularly through livestock markets, communal grazing, trade routes, and cross-border movement in regions with porous borders and informal livestock economies (4,17,67). In market-oriented production systems, frequent movement between farms, markets, and slaughter points creates repeated opportunities for viral dissemination and multi-focal outbreak initiation (40,67). Network-based approaches and outbreak investigations consistently indicate that high-connectivity nodes—markets, traders, and transport systems—serve as amplifiers of transmission, making movement control a critical component of outbreak prevention and response (67,40).

Environmental conditions further influence FMD dynamics. FMDV survives longer in cool and moist climates, and seasonal increases in outbreak risk have been associated with wetter periods and temperature conditions that favor viral stability and aerosol spread (23,73). In addition to direct animal-to-animal transmission, indirect spread through contaminated vehicles, equipment, feed, and personnel remains a well-established driver of within-farm and between-farm transmission, particularly when biosecurity infrastructure is weak (23,99). Airborne transmission also contributes to rapid spread in specific contexts, especially in intensive systems and during high-density outbreaks (16,73).

Wildlife reservoirs and livestock–wildlife interfaces can complicate control in several regions. Evidence from East Africa demonstrates that African buffalo may maintain viral circulation and contribute to transmission at the wildlife–livestock boundary, where co-grazing and shared water sources occur (72). Similarly, surveillance conducted at interface zones has identified circulation of multiple pathogens including FMDV in cattle, buffaloes, and goats, reinforcing the need to integrate wildlife considerations into endemic-area control strategies (91).

Molecular epidemiology and lineage circulation

The application of molecular epidemiology has transformed understanding of FMDV spread and lineage persistence. Phylogenetic and lineage-tracking studies provide evidence that viral circulation is shaped by regional connectivity, animal movements, and periodic introductions across borders (27,70). For example, molecular epidemiological investigation in the Emirate of Abu Dhabi identified FMDV circulation in sheep, goats, and Arabian oryx, with two distinct lineages (SA-2018 and PanAsia-2) detected simultaneously, and animal movement implicated as the principal source of introduction and spread (31). Such findings emphasize that even in areas with structured veterinary services, multi-host transmission and interspecies circulation may sustain outbreaks when animal movement is not adequately regulated (31).

Genomic surveillance approaches, including the use of slaughterhouses as sentinel sampling points, have been proposed as cost-effective strategies for monitoring circulating lineages and supporting early outbreak detection and vaccine strain updates (39). Emerging sequencing platforms such as nanopore sequencing have further enabled rapid characterization of field samples, supporting both outbreak investigation and antigenic matching decisions (17). Collectively, these tools have become increasingly important for responding to the rapid evolutionary dynamics of FMDV, including recombination and lineage turnover (27,32).

Burden of disease: seroprevalence, morbidity, and mortality patterns

FMD burden is typically characterized by high morbidity and variable mortality depending on host species, age, and immune status. Adult animals often experience lower mortality, but productivity losses (milk reduction, weight loss, fertility impact) can be substantial and persistent, creating high cumulative economic costs at the farm and national levels (36,14). In contrast, young ruminants may experience markedly higher fatality due to acute myocarditis, which can occur with minimal external lesions and can lead to sudden unexpected death, making detection and outbreak reporting more difficult (26,104).

Seroprevalence patterns in endemic areas often reflect repeated exposure and/or vaccination. For instance, in Ethiopia, reported seroprevalence varies widely across settings, ranging from approximately 4–11% in small ruminants and 5.6–72.1% in cattle, illustrating strong heterogeneity across geographic regions, management systems, and surveillance methodologies (80,102). Similar heterogeneity has been reported across South and Southeast Asia, where multiple serotypes and sub-lineages circulate and where movement-driven transmission remains a major risk factor (22,57,73). The economic consequences of these epidemiological patterns are significant. Farm-level and national-level analyses consistently demonstrate that losses arise not only from clinical disease and productivity decline but also from costs of vaccination, surveillance, movement restrictions, and trade disruption (14,36,60). In endemic settings where culling is not feasible due to socioeconomic constraints and dependence on livestock as household capital, sustained circulation may occur despite routine vaccination, particularly when coverage is incomplete or vaccine matching is suboptimal (102,60).

Implications for surveillance and control

The epidemiology of FMD underscores that control requires a systems-based approach integrating surveillance, movement management, and vaccination strategies tailored to local context. In endemic regions, sustained vaccination programs with effective strain matching, combined with movement regulation and farmer engagement, remain essential for reducing outbreak frequency and economic losses (35,38). In FMD-free regions, preparedness depends on rapid detection, movement controls, and strong contingency planning—including consideration of vaccinate-to-live strategies, which require careful management of persistently infected animals and associated economic implications (95).

Overall, the multifactorial drivers of FMD—high transmissibility, environmental persistence, multiple serotypes without cross-immunity, animal movement networks, and wildlife interfaces—explain why eradication remains difficult and why strengthening molecular surveillance and field-applicable diagnostics is increasingly central to future control strategies (27,31,39).

CLINICAL PRESENTATION AND DIFFERENTIAL DIAGNOSIS

Typical clinical presentation in ruminants and swine

Foot-and-mouth disease (FMD) is characterized by a short incubation period and rapid onset of systemic and mucocutaneous clinical signs, with severity influenced by host species, age, immune status, and viral strain (11,12). In most outbreaks, the earliest manifestations include pyrexia, dullness, and anorexia, followed by hypersalivation and the development of vesicles that rupture to form erosions and ulcers on the tongue, dental pad, buccal mucosa, and lips (32,77). Vesicular lesions also occur on the feet—particularly the interdigital space, coronary band, and heel bulbs—resulting in lameness and reluctance to move, which may be pronounced in cattle and small ruminants (57,77). Secondary bacterial infection of ruptured vesicles may exacerbate lesions, prolong recovery, and increase welfare concerns (77,92). In swine, lesions on the feet often predominate, and lameness may be severe; pigs can act as amplifying hosts due to high levels of aerosolized virus shedding, increasing the risk of rapid herd-to-herd spread in intensive systems (16,18). In small ruminants, clinical signs may be mild or subclinical compared with cattle, but infection still contributes to transmission and can drive persistent endemicity when outbreaks are not detected promptly (80,102).

Young ruminants may develop acute myocarditis, sometimes without visible oral or foot lesions, leading to sudden death ("tiger heart") and potentially masking the true extent of infection during early outbreak phases (26,104). At the herd level, even when mortality is low in adults, morbidity is typically high, and productivity losses—including reductions in milk yield, weight gain, and reproductive performance—are economically significant (14,36,60).

Clinical diagnosis and limitations

Clinical diagnosis of FMD is based on the recognition of vesicular disease patterns, history of rapid herd spread, and epidemiological context such as recent animal movement or proximity to markets (4,17). However, clinical recognition is not definitive. Multiple vesicular diseases can mimic FMD, and clinical differentiation is particularly unreliable in early infection, mild cases, vaccinated herds, or in species such as small ruminants where lesions may be subtle (28,102). Therefore, clinical suspicion should be treated as a trigger for immediate containment and laboratory confirmation rather than as a confirmatory diagnosis (25,26).

Differential diagnosis of vesicular diseases

Several transboundary and endemic vesicular diseases produce overlapping clinical signs in livestock, particularly in cattle and pigs. These include vesicular stomatitis, swine vesicular disease, vesicular exanthema of swine, and Seneca Valley virus infection. Given the severe trade and regulatory implications of an FMD diagnosis, laboratory confirmation is essential for differentiation and outbreak response (28,25).

Table 1. Key differential diagnoses of FMD and distinguishing features

Disease	Primary host(s)	Clinical similarity to FMD	Distinguishing clues (clinical/epidemiological)	Diagnostic confirmation (examples)	Notes
Foot-and-mouth disease (FMD)	Cattle, buffalo, sheep, goats, pigs; wild ungulates	Vesicles/erosions in mouth and feet, drooling, lameness, fever	Very rapid within-herd spread; severe economic/trade impact; young animals may die from myocarditis	RT-PCR/qPCR; antigen ELISA; virus isolation; sequencing	Multiple serotypes; limited cross-immunity (9,31)
Vesicular stomatitis (VS)	Cattle, horses; occasionally pigs	Vesicles in mouth, teats, coronets	Often seasonal and associated with insect vectors; horses commonly affected (unlike FMD)	RT-PCR; virus isolation; serology	Important differential in the Americas; regulatory implications (28)
Swine vesicular disease (SVD)	Pigs	Foot vesicles, lameness, snout lesions	Usually pigs only; oral lesions may be mild; outbreaks linked to swine movement	RT-PCR; virus isolation	Clinically hard to distinguish from FMD in pigs (28)
Vesicular exanthema of swine (VES)	Pigs	Vesicular lesions similar to FMD	Historically linked to marine mammal reservoirs and contaminated feed (where relevant)	RT-PCR; virus isolation	Rare/eradicated in many regions; still critical as a textbook differential (28)
Seneca Valley virus (SVV)	Pigs	Vesicular lesions on snout/feet; lameness	Often affects nursery/finisher pigs; lesions may be accompanied by neonatal mortality in some events	RT-PCR; sequencing	Increasingly recognized; easily misclassified as FMD without lab confirmation (28)

Note: Because FMD is a notifiable transboundary disease, suspected cases should trigger immediate movement restriction and laboratory confirmation using nucleic acid detection and/or antigen assays (25,26).

DIAGNOSTIC STRATEGIES FOR FOOT-AND-MOUTH DISEASE VIRUS

A robust diagnostic approach for FMD integrates clinical suspicion, outbreak epidemiology, and laboratory confirmation. In practice, diagnosis typically follows a pathway of: **(i)** clinical detection and outbreak investigation, **(ii)** targeted sample collection, **(iii)** rapid detection of viral antigen or nucleic acid, **(iv)** serotyping and/or genomic characterization, and **(v)** serology for surveillance and vaccine evaluation (25,26,33). Laboratory confirmation is essential because FMD cannot be reliably distinguished from other vesicular diseases based on clinical signs alone (28).

Sample types, timing, and handling considerations

Diagnostic yield depends heavily on the type of specimen and timing relative to disease onset. Vesicular epithelium and vesicular fluid contain high viral loads early and are preferred for antigen detection and molecular assays. Oropharyngeal (OP) fluid and throat swabs are useful for detecting persistent infection and for surveillance. Serum is essential for serology (VNT, ELISAs) and DIVA strategies using non-structural proteins (25,26,33). The choice of specimen should consider biosafety, cold chain, and laboratory capacity; in resource-limited settings, sample preservation tools such as FTA cards may facilitate safe transport while maintaining diagnostic sensitivity (3).

Table 2. Recommended specimens by diagnostic purpose

Diagnostic purpose	Preferred specimens	Typical best timing	Key notes / limitations	Key references
Rapid confirmation of suspected clinical case	**Vesicular epithelium, vesicular fluid, lesion swabs**	**Early acute phase (first few days)**	**Highest viral load; ideal for antigen ELISA and RT-PCR/qPCR**	**(25,33)**
Serotyping / molecular epidemiology	**Vesicular epithelium; OP fluid; swabs for sequencing**	**Acute phase (highest viral RNA)**	**Requires adequate RNA quality; sequencing supports lineage tracking**	**(17,31,39)**
Surveillance / detection of carriers	**OP fluid (probang), throat swabs**	**Post-acute / convalescent phase**	**Carrier detection is complex; interpretation must consider vaccination and past exposure**	**(84,95)**
Serology (infection exposure and vaccine response)	**Serum**	**≥7–14 days post-infection or post-vaccination**	**VNT is gold standard; ELISAs scalable; DIVA uses NSP assays**	**(20,38,33)**

Clinical diagnosis and field-based screening

Clinical diagnosis provides the earliest trigger for outbreak response, but it must be supported by field-appropriate screening assays when laboratory access is delayed. Point-of-care lateral flow devices and chromatographic strip tests can detect FMDV antigens within 15–30 minutes and are operationally attractive for rapid outbreak triage; however, performance varies by product, serotype, and sample quality, and some devices may not reliably differentiate all serotypes (21,33,44). For this reason, field screening should be linked to confirmatory testing in reference laboratories whenever possible (25,26).

Histopathology and immunohistochemistry (IHC)

Histopathology may support diagnosis by identifying vesicular epithelial degeneration, necrosis, and inflammatory changes, while immunohistochemistry can demonstrate viral antigen in tissue sections. Although IHC can improve specificity when appropriate reagents are available, it is generally not a first-line field diagnostic tool due to time requirements and the need for laboratory infrastructure and trained personnel (79). It is best positioned as a supportive method in research settings or retrospective confirmation when molecular diagnostics are unavailable (79).

Virus isolation and characterization

Virus isolation remains a valuable method for confirming infectious virus and generating isolates for antigenic and genomic characterization. Susceptible cell lines (e.g., BHK-21 and others) can be used, but isolation is time-consuming, costly, requires viable virus, and demands enhanced biocontainment facilities—constraints that limit routine use in many endemic settings (37,49). Nevertheless, isolation remains relevant for vaccine strain matching, experimental work, and situations where molecular results are ambiguous or require validation (37).

Serological diagnosis and DIVA strategies

Serology is essential for surveillance, estimating exposure, and monitoring vaccine-induced immunity. The **virus neutralization test (VNT)** is widely regarded as the gold standard for detecting serotype-specific neutralizing antibodies and predicting vaccine cross-protection, but it is labor-intensive and requires cell culture capacity and biocontainment (12,38). Competitive and solid-phase competitive ELISAs (SPCE) provide scalable alternatives suitable for large-scale surveillance. In addition, non-structural protein (NSP) assays targeting proteins such as **3ABC** support DIVA strategies by distinguishing infected animals from those vaccinated with purified structural antigen vaccines (33,38,79). This distinction is critical for endemic control programs and for trade-related certification in settings using vaccination (35,38).

Molecular detection: RT-PCR, qPCR, RT-LAMP, and emerging platforms

Nucleic acid amplification tests are central to modern FMD diagnosis because of their speed, high sensitivity, and ability to detect infection before visible lesions fully develop.

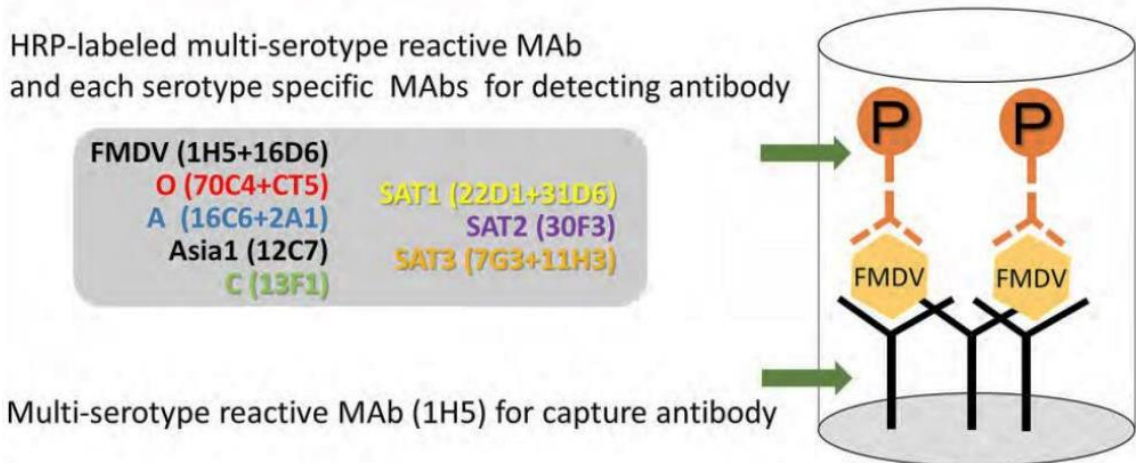

Figure 2 Sandwich ELISA

Conventional **RT-PCR** provides rapid detection and can be designed for broad detection across serotypes or for targeted typing, but assay performance may be affected by primer mismatches, contamination, and the genetic diversity of circulating strains (30,33). **Real-time RT-PCR (qPCR)** improves sensitivity, reduces contamination risk by minimizing post-amplification handling, and supports quantification of viral RNA, making it highly suitable for outbreak confirmation and surveillance (30,33). However, even qPCR assays may exhibit serotype bias and may fail to detect a small subset of divergent isolates, emphasizing the need for assay updating and periodic validation against new lineages (30,33). **RT-LAMP** offers rapid isothermal amplification without requiring sophisticated thermal cycling equipment and may be well-suited for decentralized testing, although careful validation and quality control are required to prevent false positives and to ensure robustness in field conditions (12,91). Recent diagnostic innovation has expanded to biosensor and microarray-based systems (53,79), and to CRISPR/Cas workflows that show strong potential for point-of-care detection with high specificity and low detection limits, particularly in swine settings for serotype-specific identification (68).

Table 3. Diagnostic methods for FMDV: principles, performance, and operational suitability

Method	Detects	Typical sample(s)	Turnaround time	Infrastructure needs	Serotyping ability	Strengths	Key limitations	Key references
Clinical diagnosis	Clinical syndrome	Live animal exam	Immediate	Field	No	Early suspicion; triggers containment	Not specific; overlaps with other vesicular diseases	(28,25)
Antigen capture ELISA	Viral antigen	Vesicular epithelium/fluid	Hours	Lab + cell culture often	Yes (depending on kit)	Widely used; scalable	Requires sufficient antigen load; may need culture amplification	(33,25)
Sandwich ELISA	Viral antigen	Vesicular samples	Hours	Lab	Often yes	User-friendly; can process many samples	Sensitivity depends on sample quality and antibodies used	(79,6)
Indirect sandwich ELISA (3ABC / NSP)	Antibodies to NSP	Serum	Hours	Lab	No (infection marker)	Supports DIVA; useful for surveillance	Requires validation; interpretation affected by vaccine purity	(79,38)
Solid-phase competitive ELISA (SPCE)	Antibodies	Serum	Hours	Lab	Indirect/limited	Suitable for large-scale testing	May not reflect neutralizing protection as well as VNT	(79,38)
Virus neutralization test (VNT)	Neutralizing antibodies	Serum	Days	Cell culture + containment	Yes	Gold standard; predicts cross-protection	Labor-intensive; contamination risk; high biosafety needs	(12,38)

Method	Detects	Typical sample(s)	Turnaround time	Infrastructure needs	Serotyping ability	Strengths	Key limitations	Key references
Complement fixation test (CFT)	Antigen–antibody reaction	Serum	Hours–1 day	Lab	Limited	Low-cost; historical method	Lower sensitivity; labor-intensive; largely replaced by ELISA	(48,6)
Virus isolation	Infectious virus	Vesicular samples, swabs	Days	High containment + cell culture	Yes (after typing)	Confirms viable virus; supports sequencing/vaccine matching	Slow, expensive; requires viable virus	(37,49)
Lateral flow assay (LFA)/CST	Viral antigen	Vesicular samples	15–30 min	Field	Variable	Rapid field triage	Serotyping often limited; performance varies by kit	(21,33)
RT-PCR	Viral RNA	Vesicular epithelium/fluid, swabs	Hours	Lab	Yes (if type-specific primers)	Sensitive; rapid; widely used	Primer mismatch risk; contamination; may miss divergent isolates	(30,33)
Real-time RT-PCR (qPCR)	Viral RNA	Vesicular samples, swabs	Hours	Lab	Usually no (unless multiplexed)	High sensitivity; reduced contamination; quantification	Serotype bias possible; requires equipment	(30,33)
RT-LAMP	Viral RNA	Vesicular samples, swabs	<1 hour	Low-to-moderate	Limited	Simple, rapid, minimal instrumentation	Needs careful validation; false positives possible	(12,91)
Multiplex PCR (mPCR)	Viral RNA	Vesicular samples	Hours	Lab	Yes	Differentiates serotypes in one assay	Primer design sensitive; may miss novel variants	(6)
Microarray / biosensor	Viral RNA/antigen (platform-dependent)	Various	Minutes–hours	Specialized	Potentially yes	High-throughput; innovative screening	Cost, availability, validation constraints	(79,53)
NGS / nanopore sequencing	Viral genome	Vesicular samples, swabs	Hours–days	Sequencing capacity	Yes (high-resolution)	Lineage tracking; supports vaccine strain decisions	Requires expertise, bioinformatics; cost	(17,39,31)
RT-RAA-CRISPR/Cas13a	Viral RNA	Swine samples	~1 hour	Portable platform	Yes (targeted)	High specificity; low detection limits; field potential	Still emerging; needs broader validation	(68)

Table 4. Practical diagnostic decision matrix for endemic and outbreak settings

Scenario	Primary diagnostic goal	Recommended first-line test(s)	Confirmatory / follow-up test(s)	Notes
Acute vesicular outbreak on farm (field)	Rapid triage + containment	Clinical suspicion + LFA (if available)	qPCR / RT-PCR + antigen ELISA	Field tests guide immediate action; confirm in lab (25,33)
Acute outbreak (lab access available)	Confirm infection + detect viral RNA early	qPCR / RT-PCR	Antigen ELISA + sequencing (if needed)	Sequencing supports lineage identification and vaccine matching (31,39)
Endemic-area surveillance	Estimate exposure + evaluate vaccine programs	SPCE / NSP ELISA (DIVA)	VNT for neutralizing antibody assessment	VNT reserved for targeted evaluation due to complexity (38,79)
Vaccine strain evaluation / cross-protection	Predict protection	VNT	Sequencing + antigenic characterization	Serology helps estimate cross-protection (38)
Carrier monitoring / vaccinate-to-live settings	Detect persistence + policy decisions	OP fluid sampling + RT-PCR/qPCR	Sequencing, targeted serology	Interpretation requires context; policy-relevant in FMD-free regions (84,95)

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PREVENTION AND CONTROL STRATEGIES

Principles of FMD prevention and outbreak control

Foot-and-mouth disease (FMD) control requires an integrated approach combining **early detection**, **movement restriction**, **biosecurity**, and **vaccination strategies** tailored to the epidemiological status of a country (endemic vs FMD-free) and the resources available for implementation (14,35,60). Because FMDV spreads rapidly through direct contact, contaminated fomites, and aerosols, control efforts must prioritize interruption of transmission at multiple points within the livestock production system (23,99). In practical terms, this means reducing animal mixing, strengthening farm and market biosecurity, ensuring rapid diagnostic confirmation, and implementing vaccination programs with adequate coverage and serotype matching where vaccination is part of routine prevention (38,40).

In endemic regions, repeated outbreaks are often sustained by animal movement networks, high herd turnover, incomplete vaccination coverage, and strain mismatch due to rapid viral evolution and the circulation of multiple serotypes (27,35). In contrast, FMD-free regions emphasize strict import controls, preparedness planning, rapid outbreak detection, and strong regulatory enforcement to maintain freedom from disease, with vaccination considered selectively depending on outbreak scale and policy objectives (60,95).

Biosecurity and movement control

Movement control is consistently identified as one of the most influential determinants of outbreak size and spread. Restrictions on animal movement between farms, markets, and slaughter points—particularly early in an outbreak—can substantially reduce transmission potential and prevent multi-focal dissemination (40,67). In endemic regions where movement restriction is difficult to enforce due to informal markets and cross-border trade, risk reduction may require pragmatic interventions such as regulating market entry points, disinfection of transport vehicles, movement certification, and community-based reporting mechanisms (17,81). Biosecurity measures are essential for limiting both direct and indirect transmission. These include isolation of clinically affected animals, strict cleaning and disinfection procedures, controlled access of personnel and vehicles, and careful disposal of contaminated materials (23,99). Given the ability of FMDV to persist on contaminated equipment and in cool moist environments, routine disinfection and environmental hygiene are critical components of outbreak prevention, especially in high-density systems (23).

Outbreak response strategies: endemic versus FMD-free settings

The outbreak response framework differs substantially between FMD-free and endemic settings. In FMD-free regions, the standard approach often includes rapid detection, quarantine, stamping-out (culling of infected and in-contact animals), strict movement restriction, and thorough disinfection of premises, sometimes supported by emergency vaccination (60,95). Although stamping-out is operationally effective for rapid elimination, it is associated with major economic, ethical, and social challenges, particularly when outbreaks involve large numbers of animals or occur in regions where livestock represent core livelihood assets (60,95).

In endemic settings, stamping-out is frequently impractical due to financial constraints and socio-cultural barriers; therefore, **vaccination plus movement management** becomes the main control strategy (35,102). These approaches may include repeated vaccination cycles, strategic vaccination of high-risk zones, and focused improvements in market biosecurity and surveillance (35,81). In practice, the sustainability of vaccination-based control depends on vaccine availability, strain matching, sufficient coverage, and community compliance (38,81).

Ring vaccination, which targets animals surrounding infected premises, can be effective in focal outbreaks but may be less useful during widespread epidemics where multiple transmission chains occur simultaneously and where delays in vaccine deployment reduce impact (20,60). Simulation studies indicate that vaccination policies must be designed in consideration of animal movement structure, outbreak detection delays, and national logistics capacity, particularly in geographically large livestock systems (20).

Supportive treatment, animal welfare, and antimicrobial stewardship

There is no specific curative therapy for FMDV infection in routine field practice. Management is primarily supportive and aims to reduce suffering, prevent secondary infections, and limit transmission through isolation and sanitation (92,102). Supportive care commonly includes wound and lesion management (antiseptics and topical disinfectants), hydration, and nutritional support. Antibiotics may be justified only for secondary bacterial infections and severe complications, but they do not treat the viral infection itself and should not be presented as a control strategy for viral transmission (92). Importantly, inappropriate antibiotic use during FMD outbreaks may contribute to antimicrobial resistance and should be minimized through evidence-based veterinary prescribing practices and welfare-centered treatment protocols (92). Welfare considerations are particularly important in endemic low-resource settings where culling is not feasible and animals remain within the production system during recovery.

Vaccination as prevention: operational challenges and strategic considerations

Vaccination is the backbone of preventive control in endemic regions, yet its effectiveness is shaped by several constraints. First, FMDV antigenic diversity and rapid evolution require ongoing surveillance and vaccine strain updates to maintain cross-protection against circulating field viruses (38,70). Second, conventional inactivated vaccines typically provide short-term immunity (often requiring boosters at 4–6 months in high-risk settings), which creates logistical burdens for repeated mass vaccination (35,37). Third, vaccination may prevent clinical disease but may not completely prevent infection, viral replication, or the establishment of carrier status in some animals, which has implications for surveillance and trade certification (84,95). DIVA-compatible approaches, supported by non-structural protein assays (e.g., 3ABC), can improve surveillance and control in vaccination settings by enabling differentiation between infected and vaccinated animals, thus supporting outbreak investigation, certification, and targeted response (38,79). In addition, vaccination programs must account for maternal antibodies, herd turnover, and species-specific immunological responses to optimize timing and dosing schedules (35,60).

Economic and policy considerations

Control strategies must be feasible, cost-effective, and aligned with local livestock systems. Economic analyses show that losses arise not only from disease morbidity but also from trade restrictions, control costs, and productivity decline (14,36,60). In FMD-free settings, prevention and preparedness—through surveillance and border control—are economically justified due to the very high consequences of incursion (18). In endemic settings, policy choices are shaped by limited resources and competing priorities; thus, interventions such as targeted vaccination of high-risk zones, market-based surveillance, and community engagement may be more sustainable than intensive stamping-out approaches (60,102).

Table 5. Major FMD prevention and control measures: strengths, limitations, and best-use settings

Control measure	Primary mechanism	Best-use setting	Advantages	Limitations / risks	Key references
Movement restrictions / quarantine	Reduces contact and mixing	Outbreak response (all settings)	Highly effective if early and enforced	Enforcement challenges; economic disruption	(40,67,60)
Market control and transport biosecurity	Reduces spread through trade networks	Endemic settings; outbreak periods	Targets high-connectivity "amplifiers"	Requires regulation and compliance; informal trade remains risk	(17,81,67)
Farm-level biosecurity (cleaning/disinfection, controlled entry)	Reduces indirect transmission	Routine prevention + outbreak response	Limits fomite transmission; improves containment	Requires training, supplies, and sustained behavior	(23,99)
Stamping-out (culling infected/in-contact animals)	Removes infection source rapidly	FMD-free settings; focal outbreaks	Rapid elimination; supports return to freedom	Ethical/social barriers; high economic and welfare costs	(60,95)
Emergency vaccination (reactive)	Builds immunity during outbreak	FMD-free settings when outbreak enlarges	Reduces spread when culling alone insufficient	Delay to onset of immunity; requires matched vaccine	(20,60)
Routine vaccination (prophylactic)	Establishes herd immunity	Endemic regions	Most practical long-term strategy	Short duration immunity; strain mismatch; booster needs	(35,37,38)
Ring vaccination	Creates immune buffer	Localized outbreaks	Efficient resource use	Limited effect if outbreak widespread or delayed	(20,60)
DIVA-based surveillance (NSP assays)	Distinguishes infected vs vaccinated	Vaccination settings	Strengthens monitoring and certification	Requires validated diagnostics; depends on vaccine purity	(38,79)
Wildlife interface management (reduce contact)	Reduces spillover/maintenance	Wildlife–livestock interface zones	Can lower reintroduction risk	Logistically difficult; ecological constraints	(72,91)
Supportive welfare-centered management	Reduces suffering, secondary complications	Endemic settings	Improves welfare; may reduce prolonged losses	Does not prevent transmission; requires guidance	(92,102)

Table 6. Comparative outbreak-control policies in FMD-free versus endemic regions

Policy dimension	FMD-free setting (typical approach)	Endemic setting (typical approach)	Practical implication	Key references
Primary goal	Rapid elimination + restoration of freedom	Reduction of incidence and economic impact	Strategies differ fundamentally by feasibility and resources	(60,102)
Core interventions	Stamping-out, strict movement bans, surveillance, disinfection	Routine vaccination, movement management, surveillance	Endemic settings rely more on vaccination and incremental control	(60,35)
Role of vaccination	Often emergency/reactive; sometimes avoided for trade status	Routine/prophylactic; boosters required	Requires vaccine matching and sustained coverage	(38,35,37)
Surveillance emphasis	Early detection + trace-back/tracing networks	Continuous monitoring + serology (DIVA where possible)	Endemic settings require ongoing surveillance to guide strain updates	(39,38)
Carrier state management	Critical concern in vaccinate-to-live strategies	Often tolerated due to repeated exposure	Influences trade, certification, and long-term eradication plans	(84,95)
Socioeconomic constraints	High resources but high trade consequences	Strong dependence on livestock; limited capacity for culling	Policy choices shaped by livelihood preservation	(60,102)
Operational feasibility	High enforcement capacity	Variable enforcement; informal movement common	Movement control may require community-based enforcement models	(81,17)

Table 7. Practical recommendations for control program design in endemic settings (implementation-focused)

Program component	Key recommendation	Rationale	Implementation note	Supporting references
Vaccination program	Use multivalent vaccines aligned to circulating serotypes; schedule boosters	Cross-protection depends on antigenic match; immunity short-lived	Integrate strain surveillance into annual vaccine updates	(35,38,70)
Movement and markets	Strengthen market entry control, movement certification, vehicle disinfection	Markets amplify spread	Prioritize high-risk corridors and "hub" markets	(67,17,81)

Surveillance	Combine outbreak reporting, slaughterhouse sentinel sampling, and serology	Detect lineages early and guide vaccine matching	Add DIVA where vaccination occurs	(39,38,79)
Diagnostics	Deploy rapid field screening linked to lab confirmation	Early containment depends on speed	Ensure sample logistics and reference lab linkage	(25,33)
Farmer engagement	Improve awareness and reporting	Reporting delay enlarges outbreaks	Use targeted education and incentives	(81,13)
Welfare and AMR	Restrict antibiotics to secondary infections; standardize lesion care	AMR risk; welfare priorities	Provide veterinary treatment protocols	(92)

IMMUNIZATION AND VACCINE PLATFORMS

Role of immunization in FMD control

Immunization remains a cornerstone of foot-and-mouth disease (FMD) control, particularly in endemic regions where stamping-out policies are often impractical due to socioeconomic constraints, high livestock dependence, and limited compensation capacity (60,102). Vaccination reduces clinical disease, limits virus shedding, and contributes to herd immunity when adequate coverage is achieved; however, vaccine effectiveness is heavily dependent on antigenic matching between vaccine strains and circulating field viruses, the vaccination schedule, cold-chain integrity, and the immune status of target populations (35,38). The presence of seven serotypes and substantial intra-serotype variation—with limited cross-protection—requires continuous molecular and antigenic surveillance to inform vaccine selection and updates (9,38,70).

In FMD-free settings, vaccination is generally used as an emergency tool to support outbreak containment (e.g., ring vaccination or strategic vaccinate-to-live) when culling alone is insufficient or when political, ethical, or logistical constraints limit stamping-out (20,95). In such contexts, vaccination decisions must balance outbreak containment benefits with downstream implications for surveillance, trade resumption, and the management of potentially persistently infected animals (95).

Inactivated (killed) whole-virus vaccines: current standard

Inactivated whole-virus vaccines represent the most widely used prophylactic platform globally and remain the standard for routine vaccination in endemic regions (14,63). These vaccines are commonly formulated with aluminium salts, aqueous formulations, or oil-emulsion adjuvants and are designed to induce neutralizing antibodies that reduce clinical disease and transmission (63,69). Vaccination schedules typically include a primary series (often two doses approximately one month apart), followed by boosters at intervals that may vary based on risk and epidemiological context (e.g., every 4–6 months in high-risk zones) (35,69).

Despite their widespread use, inactivated vaccines have recognized limitations: (i) relatively short duration of immunity, necessitating repeated boosting; (ii) reduced effectiveness when antigenic match is poor; (iii) demanding manufacturing requirements and biosafety constraints due to production from live virus; and (iv) incomplete prevention of infection and potential persistence, meaning vaccinated animals may still become infected and contribute to transmission under some circumstances (37,51,84). These limitations underscore the need for improved vaccine design and program implementation, particularly where multiple serotypes co-circulate and vaccine coverage is inconsistent (38,70).

DIVA-compatible strategies and marker vaccines

DIVA (differentiating infected from vaccinated animals) approaches are essential for enhancing surveillance and enabling evidence-based trade and control policies in vaccinated populations. DIVA is generally supported through non-structural protein (NSP)-based assays (e.g., 3ABC) because purified inactivated vaccines ideally contain structural proteins but minimal NSP content, whereas infected animals develop antibodies to both (38,79). The utility of DIVA depends on vaccine purity, assay validation, and strong surveillance infrastructure. When implemented effectively, DIVA supports outbreak investigation, identification of silent infection, and strategic management of vaccinated herds (38,79).

Next-generation vaccine platforms

Recognizing the limitations of conventional inactivated vaccines, multiple next-generation platforms are under development, aiming to improve safety, immunogenicity, and breadth of protection. These include peptide-based and multi-epitope vaccines, virus-like particle (VLP) vaccines, recombinant subunit vaccines, viral vector-based vaccines, and genetically engineered attenuated candidates (34,63,66). Peptide-based approaches offer improved biosafety and manufacturing flexibility but may require strong adjuvants and optimized delivery systems to generate robust protective immunity (34). VLPs mimic natural viral structure without containing infectious genome material, offering strong immunogenicity with improved safety profiles, though large-scale production and stability remain major challenges (78).

Live-attenuated vaccination strategies have historically been constrained by safety concerns, particularly the risk of reversion to virulence. Modern genetic approaches attempt to reduce this risk through targeted deletions (e.g., leader protease modifications) and stabilization strategies, yet careful evaluation remains necessary due to biosafety implications in livestock populations (6). Viral vectors represent another promising approach by delivering immunogenic FMDV proteins to induce humoral and cellular responses, though their effectiveness may vary by species and platform, and pre-existing vector immunity can limit performance (6).

Adjuncts, immune modulators, and combined vaccination approaches

Emerging evidence suggests that adjunct immunomodulators may enhance protection when used with vaccination, potentially improving early outbreak control. For example, quercetin has been reported to reduce viral propagation and to enhance immune response markers in experimental models, with improved outcomes when combined with vaccination, indicating potential as an adjunct in outbreak settings (56). Combination vaccines targeting co-circulating pathogens (e.g., FMD and haemorrhagic septicemia) have also shown favorable immune responses under field conditions, offering potential programmatic advantages, particularly in developing livestock systems (69).

In addition, interferon-based biotherapeutics and antivirals have been investigated as supportive measures to limit replication and enhance host antiviral defenses, though translation into routine field application remains limited by cost, logistics, and regulatory constraints (66).

Nanotechnology and advanced adjuvant platforms

Nanotechnology approaches have been increasingly explored for both diagnosis and immunization. Nano-adjuvants such as nanoliposomes, layered double hydroxide nanoparticles (LDH NPs), ferritin nanoparticles, and nano-emulsion systems have demonstrated potential to enhance both humoral and cellular immune responses, provide slow-release antigen delivery, and improve immunogenicity with reduced toxicity compared with some conventional oil emulsions (24,78,94). For example, LDH NPs have shown adjuvant potential in pigs and mice by sustaining antibody responses, suggesting a slow-release immune stimulation effect that may be valuable for long-term immunity (94). Similarly, nano-emulsion adjuvants based on squalane have demonstrated enhanced immune responses and biocompatibility in VLP vaccine systems, indicating possible scalability for future vaccine development (78). Nevertheless, many nano-adjuvant systems remain at preclinical or early translational stages, and their deployment in livestock vaccination programs will require clear evidence of safety, field performance, manufacturing feasibility, and cost-effectiveness (78,94).

Table 8. Vaccine platforms for FMD: comparative evaluation, advantages, limitations, and development stage

Vaccine platform	Core immunological mechanism	Advantages	Limitations	Safety considerations	Development / use status	Key references
Inactivated whole-virus vaccines	Neutralizing antibody induction	Widely available; proven field effectiveness; scalable	Short-lived immunity; strain matching required; may not prevent infection/carrier state	Requires high biosafety manufacturing; cold chain	Routine use (endemic settings)	(63,35,38)
Multivalent inactivated vaccines	Serotype coverage expansion	Better coverage where multiple serotypes circulate	Antigen competition; still requires matching; boosters needed	Similar to inactivated vaccines	Routine use in many endemic programs	(35,102)
Peptide-based vaccines	Targeted epitope immunity	Biosafe; easier manufacturing	Often lower immunogenicity; needs strong adjuvant/delivery	High safety profile	Experimental / early development	(34)
Virus-like particles (VLPs)	Mimics virion structure without genome	Strong immunogenicity; safe	Production complexity; stability constraints	High biosafety profile	Experimental; increasing interest	(78)
Recombinant subunit vaccines	Antigen-specific humoral/cellular immunity	Safe; adaptable	Needs adjuvant and optimized delivery	Safe; no infectious virus	Experimental	(24,63)
Viral vector vaccines	Vector-mediated antigen expression	Strong cellular + humoral responses	Vector immunity; variable species performance	Depends on vector	Experimental	(6)
Genetically attenuated vaccines	Replication-limited immune priming	Potential rapid protection	Reversion concern; regulatory constraints	Higher risk than non-replicating	Experimental; cautious development	(6)
Marker vaccines + DIVA strategy	Enables infection/vaccination distinction	Improves surveillance and trade certification	Depends on vaccine purity and validated assays	Safe when non-replicating	Increasing adoption with NSP assays	(38,79)
Nano-adjuvant enhanced vaccines	Improved antigen delivery and immune activation	Stronger, more durable responses; reduced adverse effects	Cost and manufacturing complexity; field validation needed	Safety must be proven per platform	Preclinical/early translational	(78,94,24)

Table 9. Programmatic considerations for FMD vaccination (implementation-focused)

Program factor	Why it matters	Best practice recommendation	Evidence / support
Strain matching	Determines protection and cross-protection	Align vaccine strains with regional lineages via surveillance	(38,70,27)
Booster schedule	Immunity is short-lived	Boost at 4–6 months in high-risk settings; align with seasonality	(35,37)
Coverage threshold	Low coverage maintains transmission	Target high coverage in all susceptible species	(35,60)
Cold chain integrity	Affects potency	Strengthen storage and distribution monitoring	(69,63)
Maternal antibodies	Interfere with early vaccination	Schedule vaccination to avoid maternal antibody interference	(35,60)
DIVA integration	Improves monitoring and outbreak detection	Use NSP assays (e.g., 3ABC) in vaccinated populations	(38,79)

EMERGING TECHNOLOGIES AND FUTURE DIRECTIONS

Genomic surveillance and phylogenetics for outbreak management

Genomic surveillance has become increasingly central to understanding FMDV circulation, lineage emergence, and vaccine match requirements. Phylogenetic analysis of field isolates allows tracking of viral introductions, identification of transmission drivers, and characterization of circulating lineages, thereby supporting targeted control interventions (27,31). The emergence and extinction of FMDV lineages are shaped by ecological and evolutionary drivers, and genomic tools provide insight into these processes, particularly in regions where cross-border movement

is common (27). Sequencing technologies—including nanopore sequencing—can be applied directly to clinical samples to accelerate characterization of outbreak strains, enabling faster decisions regarding vaccine updates and improving evidence-based outbreak response (17). Sentinel sampling strategies, such as using slaughterhouses as surveillance points, may offer practical pathways for routine genomic monitoring in endemic regions, where large-scale farm-based sampling may be resource-intensive (39).

Point-of-care diagnostics and rapid field detection

Field-applicable diagnostics remain a priority because diagnostic delays can substantially increase outbreak spread. Lateral flow assays offer rapid screening, but emerging platforms such as RT-LAMP and CRISPR/Cas diagnostics provide improved sensitivity and specificity, with reduced dependency on centralized laboratories (12,68). Recent work has demonstrated that RT-RAA-CRISPR/Cas13a platforms can detect swine FMDV serotype O with high specificity and low detection limits within approximately one hour, highlighting their potential for outbreak containment in intensive swine systems (68).In the longer term, integration of portable biosensors, microarray-based detection, and simplified sequencing workflows may enable decentralized testing and faster linkage between detection and control action, particularly in regions where laboratory access is limited (53,79).

Vaccine innovation and long-lasting immunity

Next-generation vaccine development is increasingly focused on achieving broader antigenic coverage, stronger cellular immunity, and longer-lasting protection. Advances in epitope mapping, antibody neutralization studies, and rational antigen design may strengthen the ability to predict cross-protection and reduce the frequency of booster vaccination (42,38). Nanotechnology-enhanced vaccine delivery platforms may also contribute to more durable immunity through sustained antigen release and optimized immune stimulation, although field validation and manufacturing scalability remain limiting factors (78,94).

Priority research gaps

Despite major advances, several critical gaps remain. These include:improved prediction of vaccine cross-protection against evolving field strains through standardized serology and antigenic cartography approaches (38); development of affordable and robust point-of-care diagnostics suitable for low-resource endemic settings (12,68); enhanced understanding and management of persistent infection and its implications for vaccinate-to-live strategies (95,84); and scalable vaccine platforms that provide durable immunity across multiple serotypes while remaining cost-effective and safe for widespread livestock use (63,78).

CONCLUSION

Foot-and-mouth disease (FMD) remains a major constraint to livestock productivity and international trade due to its high transmissibility, environmental persistence, and circulation of multiple antigenically distinct serotypes. Disease dynamics are strongly shaped by animal movement networks, market connectivity, seasonal influences, and livestock–wildlife interfaces, particularly in endemic regions across Asia, Africa, and the Middle East (17,27,72). While clinical recognition is essential for early suspicion, laboratory confirmation is mandatory because FMD cannot be reliably distinguished from other vesicular diseases without antigen or nucleic acid detection (28,33). Molecular diagnostics, including RT-PCR and qPCR, remain central for rapid confirmation, while serological approaches and DIVA-compatible NSP assays strengthen surveillance in vaccinated populations (30,38,79).

Prevention and control depend on coordinated biosecurity and movement management, combined with vaccination strategies that are tailored to local epidemiology and supported by continuous strain surveillance. In endemic settings, routine multivalent vaccination with adequate boosting and coverage is essential, whereas FMD-free regions rely primarily on rapid containment and stamping-out policies, with emergency vaccination used selectively to reduce outbreak spread (35,60,95). The limitations of conventional vaccines—including short duration of immunity and incomplete prevention of infection—support ongoing development of next-generation vaccine platforms, nano-adjuvants, and adjunct immunomodulatory approaches (63,78,94). Future progress in FMD management will increasingly depend on integrated genomic surveillance, rapid point-of-care diagnostics, and evidence-based vaccine matching supported by molecular epidemiology. Strengthening diagnostic access in endemic regions, improving vaccine durability and cross-protection, and addressing the practical and economic challenges of large-scale implementation remain key priorities for reducing the global burden of FMD and supporting sustainable livestock production systems (17,38,68).

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