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Foot-and-Mouth Disease (FMD) Current and Novel Vaccines: Progress and Limitations

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ABSTRACT

Foot and mouth disease (FMD) is one of the viral contagious diseases that cause severe economic losses worldwide. Vaccination is the cornerstone method in controlling this disease in Egypt and worldwide. Although the current inactivated vaccines are widely used in controlling the disease. Vaccination failure is a common concern. Attenuated, marker-inactivated, recombinant protein vaccines, synthetic peptides, and empty capsid vaccines are a future pathway. The primary objectives of this paper are to describe the current foot-and-mouth disease vaccines with special reference to Egypt and review the literature on current vaccine limitations and future vaccines.

Keywords: Foot and mouth disease; Vaccine; Inactivated vaccine; Peptide vaccine; Plant based vaccine ; Virus like proteins ; DNA vaccine ; Adjuvant

INTRODUCTION

Foot-and-mouth disease (FMD), a viral animal illness that kills young animals reduces adult animals' productivity, and has severe economic and social repercussions, is still a source of concern in nations with highly developed animal husbandry (Jamal & Belsham, 2013). It is a transboundary disease with a high contagiousness that affects mainly cloven-hooved animals including cattle, buffalo, swine, sheep, and goats, as well as around 70 different species of animals with cloven hooves in the wild (Jamal & Belsham, 2013). Although cattle serve as the primary host, some strains can also infect swine. The foot-and-mouth disease virus (FMDV) infection is the disease's primary cause (Carolina Stenfeldt et al., 2014).

FMDV firstly invades the swine oro-pharynx (Carolina Stenfeldt et al., 2014) (cattle nasopharynx) then spreads systemically, creating vesicles in the mouth, interphalangeal space, the udder, teats, and foot (Jonathan Arzt et al., 2010). Aside from having a high body temperature, infected animals often exhibit clinical signs including excessive salivation and decreased milk yield. They become more susceptible to secondary illnesses and lose some weight, which reduces their output over the long run (Jonathon Arzt et al., 2011).

Although FMD disease is easier to transmit from one animal to another, mortality is scarce due to the infection often goes away in two weeks. Young affected animals may die due to myocarditis, or heart muscle degeneration, the majority of the time (Jonathon Arzt et al., 2011). Due to the low amounts of infectious virus dose present in the oropharynx of affected buffalo, cattle, and sheep (but not pigs), infection may

continue after the acute stage (Carolina Stenfeldt & Arzt, 2020). The foot-and-mouth disease (FMD), which affects livestock with cloven hooves, has a severe impact on the global economy (Do et al., 2022).

Due to its endemicity in poor nations, FMD poses a serious threat to industrialized nations' claim as FMD-free. Controlling the FMD virus has thus far been a crucial need (Do et al., 2022). Foot-and-mouth disease (FMD) affects animals that are cloven-hooved and is extremely contagious and economically damaging (Chathuranga et al., 2022).

Traditional use of inactivated vaccinations is given to the susceptible animals of disease-endemic nations to immunize them against the FMD virus (FMDV). However, the inactivated FMD vaccination has several drawbacks, including security issues(Chathuranga et al., 2022).

Subunit proteins have been investigated as potential substitute vaccination candidates to get around these restrictions (Chathuranga et al., 2022). Although all animals susceptible to FMD are vaccinated with traditional vaccines, FMD outbreaks continue (Park et al., 2021). The inactivated FMD vaccines are currently effective against FMDV, However, have many significant limitations that include high bio-safety manufacturing needs, the low thermal stability of the antigens, the likelihood of incomplete the inactivation process, and the possibility of virus leakage from the production facilities and the remaining nonstructural proteins which makes it too difficult to differentiate between the infected and the vaccinated animals (DIVA) (Cao et al., 2014).

Therefore, a variety of research techniques have been investigated to create substitute innovative vaccines to solve these deficiencies (Xiao et al., 2016).

1. Foot and mouth disease

One of the problems that significantly affected the cattle sector was foot and mouth disease (FMD). Animals with split-hooved feet, such as cattle, sheep, water buffalo goats, pigs, and wild animals, are susceptible to catching this disease (Carolina Stenfeldt et al.,2018). The affected animals also get vesicles in their mouths, hooves, and teats in addition to having a high fever. Animals who have these vesicles ruptured might suffer severe pain and lameness. It can easily spread to the infected animals through contact with contaminated farming tools, vehicles, feed, and clothing (Carolina Stenfeldt et al., 2018).

Effective disease control strategies include vaccination, trade restrictions, quarantines, surveillance, and culling of both affected and healthy (uninfected) animals (Carolina Stenfeldt et al., 2018). Due to its impact on the worldwide commerce of live animals and its products that come from endemic nations, FMD is known to cause significant economic losses in direct and indirect ways, so badly affects both the national and the international (Cairns et al., 2017).

At the national one, FMD has an impact on the economies of those endemic nations since otherwise the limited resources are committed to surveillance programs, vaccination campaigns, restrictions on the transportation of animals, and the closure of all animal markets(Jemberu et al., 2014; Knight-Jones & Rushton, 2013; Tadesse et al., 2020).

FMD is characterized at the farmer level by a high morbidity, loss in productivity, and increasing expenses for treatment and veterinary services. Furthermore, FMD can result in significant mortality in newborn calves that are still nursing because of a unique heart condition called tiger heart (Jemberu et al., 2014; Knight-Jones & Rushton, 2013; Tadesse et al., 2020).

1.1.Foot and mouth disease virus

The (FMDV) virus, which is a virus of the Aphthovirus genus, is in the cause from Picornaviridae family. Seven genetically and antigenically different serotypes of the virus are known to exist: O, C, A, Asia 1, and the Southern African Territories

(SAT) (1-3).Each serotype has many subtypes within each serotype of the virus (Racaniello, 2001). The FMDV genome, which is more than (8,000) bases in length, includes a huge open reading frame (ORF) that encodes a polyprotein that will be processed later into polypeptides. The structural proteins of the viral icosahedral capsid (VP1, VP2, VP3, and VP4) are all encoded by the genes named (1D, 1B, 1C, and 1A) respectively (Mason et al., 2003). The non-structural proteins of the capsid are encoded by the genes named (2A, 2B, 2C and 3A, 3B, 3Cpro, 3Dpol, and Lpro) (Mason et al., 2003), which are mainly the responsible for maturation and replication of the FMDV (Carrillo et al., 2005). Also, 3′ and 5′and the untranslated regions (UTRs) are very important for the process of replication and the translation of FMD viral genomes (Carrillo et al., 2005) (Figure 1).

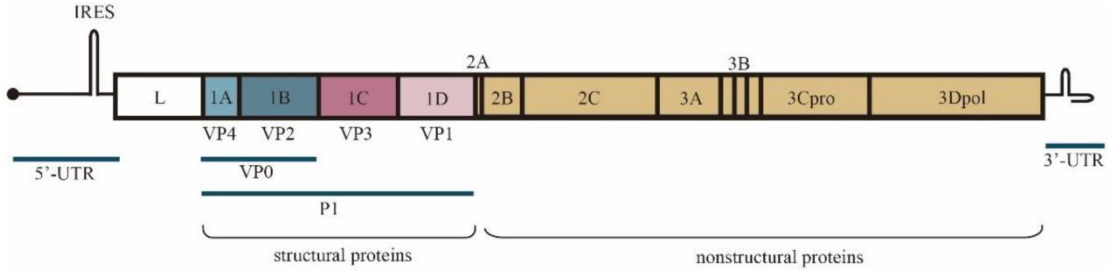

Figure1. Production and the processing of (FMDV) proteins **(Lu et al., 2022).**

The virus of foot-and-mouth disease has 7 different serotypes: A, C, O, Asia-1, and SAT (1-3). This diversity is a result of the virus' high degree of variability (Samuel & Knowles, 2001). Continuous emergence of and transmission cycles that may affect numerous nations, FMDV different serotypes are likely to have a propensity to repeat within a given geographic area. As a result, the OIE (World Organization for Animal Health) has likewise divided epidemic zones into 7 (seven) divisions (Figure 2). Each serotype is geographically restricted and indigenous to its particular location, except Asian-1 (Samuel & Knowles, 2001).

The SAT-2 serotype, which had recently become endemic in significant areas of Egypt, is one example of how serotypes might spread to different areas. The geographic range of serotypes O and A is extensive. Serotype O infections, however, have very sometimes occurred in recent years, and serotype A infections have considerably declined (Valarcher et al., 2008).

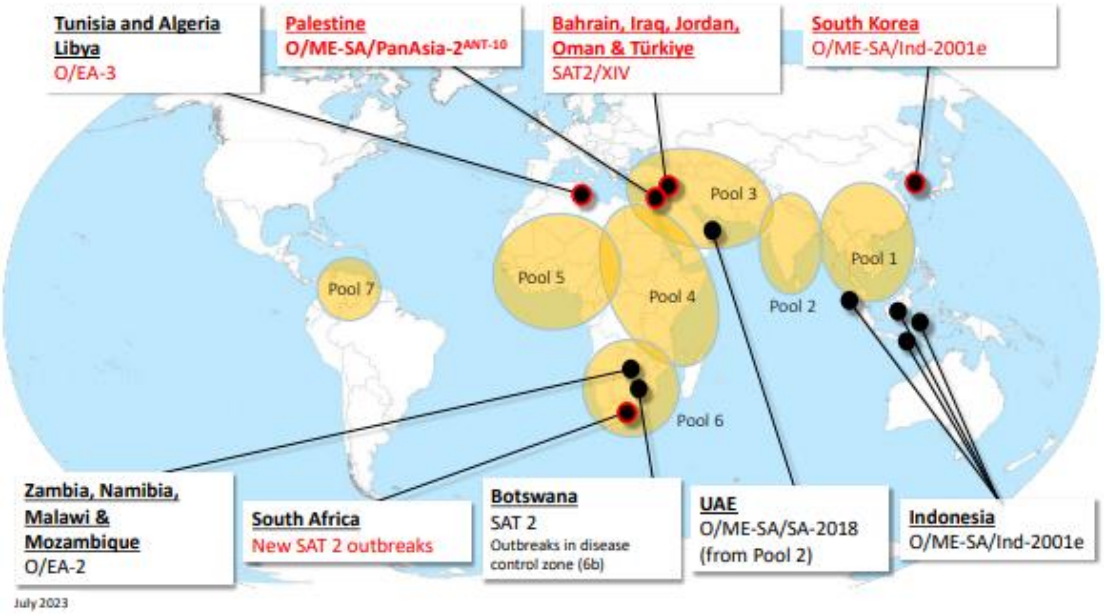

Figure2. : Recent FMD universal outbreaks (newest headline events reported April to June 2023 are highlighted in the colorred) with endemic pools highlighted in the color orange. Source: WRLFMD. Map conforms to the United Nations World Map, June 2020.

In several regions of Asia, a significant part of Africa, and the Middle East, foot-and-mouth disease is currently endemic. The use of conventional vaccines that chemically inactivate the virus has rendered foot-and-mouth disease extinct in Australia, New Zealand, and Indonesia, which are referred to as "foot-and-mouth disease-free countries"(Carolina Stenfeldt et al., 2016).

Due to the great potential antigenic variety of the virus, some nations choose not to vaccinate to keep their status as foot-and-mouth disease-free nations and to encourage the commerce of animal trade and their products. However, also FMD is a transboundary animal disease (TAD) that might appear in any normal free zones (Carolina Stenfeldt et al., 2016).

Nations need to reintroduce new vaccines after an epidemic to stop the disease's spread. However, this is uncertain and not without risk. We all know that vaccination will likely provide better protection the earlier it is administered before an outbreak (Casey-Bryars et al., 2018).

1.2. Vaccines and vaccination

To prevent FMDV sever effects, vaccination is a critical technique (Diaz-San Segundo et al., 2017). The type and quality of the vaccinations that are accessible are the most crucial components of a vaccination-based control program. With more than two billion doses manufactured each year, the Foot and Mouth Disease vaccine is the first animal vaccine to be created during the early 19th century, and also one of the most commonly used of all animal vaccinations (Knight-Jones & Rushton, 2013). The current vaccinations only protect against widespread clinical illness and do not prevent primary infection. Animals that have received the vaccine are exposed to FMDV, which causes invasion without the emergence of bad signs. Animals frequently later became the carrier, in which they silently shed the virus (C Stenfeldt et al., 2016). But we still need a vaccine that is extremely effective and secure (Lombard & Füssel, 2007).

1.2.1 History of foot and mouth disease vaccination

The earliest known method of providing active protection to a herd of cattle was to undertake "aphtization" as soon as any

case of FMD was detected in the herd or the neighbor herds. By rubbing their muzzles or the lips of animals with field virulent saliva obtained from animals with lesions of FMD, all the animals in the herd were simultaneously immunized, and this resulted in a too-early, powerful, and their also post-infectious immunity that lasted for a longer time (Blancou, 2003; Joubert & Mackowiak, 1968).

The disease appeared clinically similar to the ordinary disease but differed from it in there were many good aspects like the briefness of the clinical symptoms, the synchronization of infection in the entire herd, the absence of virulence aggravation by the passages, and lastly the goal of the operation, the immunity (monovalent) provided for many years (Blancou, 2003; Joubert & Mackowiak, 1968).

1.2.2 Pioneers

The administration of immune serum for the prevention or treatment of FMD signs in cattle came next, immediately before to the application of the vaccine. This innovative preventive strategy to safeguard herds was invented by Friedrich Löffler, who was the FMD agent's filterable nature co-discoverer (1897), and later advanced by numerous other researchers (Blancou, 2003). Many European nations after the 1st World War organized the cattle immune serum production on an industrial scale. For instance, records show that in the 1920s, 112,000 liters of the immune serum were also used in Denmark over 9 years and that about 13,000 animals were treated by it in France in a single year (Joubert & Mackowiak, 1968).

The use of immune serum in conjunction with aphtization was advocated by many authors as a way to lessen the effects of the vaccinated sickness, which is noteworthy to note (Joubert & Mackowiak, 1968). Researchers from France Vallée, Carré, and Rinjard made the first known attempt to use a vaccine to protect against FMD in 1926 (Vallée et al., 1926). The impact of formaldehyde on several infectious disease pathogens has been studied since 1922.

A report on the 1st animal true vaccine, which was created by grounding up FMD mucosal lesions in a saline buffer and inactivated at 20°C for 4 - 7 days with 0.5% formaldehyde, was published in 1925. Although the protection provided was sporadic, it was rated as adequate by then-prevailing standards when it was. By utilizing aluminum hydroxide gel concurrently, Schmidt finished the laboratory procedure in Denmark in 1932. Then the method was enhanced further by a group led by Prof. D. Waldmann, and semi-industrial production of the FMD vaccination started (Lombard et al., 2007) (Waldmann et al., 1937), In a report they released, they emphasized the good effects of a few critical elements, such as guaranteeing a pH less than 9 during the process of inactivation, utilizing a small amount of formaldehyde concentration (0.05%), plus the material kept at a higher temperature (25°C) for 2 days (Lombard et al., 2007).

As a result, the 1st contemporary method for converting FMD virus to vaccine antigens was created. It was utilized for almost fifty years, up till the 1970s, when tries were made to employ different inactivates in industrial manufacturing, such as glycidaldehyde or aziridines (Lombard et al., 2007) .

1.2.3 Industrial development

Once the challenging process of changing the dangerous (FMD) viruses into harmless antigens had been accomplished, the next challenge was to find enough virus material for vaccine manufacture. Waldmann's approach, a novel technique for gathering higher amounts of infectious material, was created to solve this issue (Lombard et al., 2007).

This method was still in use in South America in the 1970s. In Europe, it has been in use since the 1950s (Joubert & Mackowiak, 1968). The virulent material is collected, following the method, from sick cattle that are maintained in a small stable, simultaneously inoculated at various sites on the tongue, and killed

when lesions of the tongue are at their bad(Lombard et al., 2007).

To gather lymph and epithelial lesions, each tongue is isolated and scraped. Before inactivation, the pathogenic lesions of the tongue are crushed in a saline buffer, then centrifuged and diluted. One commercial cow dose of the monovalent vaccination required a volume of 60 ml during the beginning of the method's development, and each cattle tongue could produce 40 to 50 such doses(Lombard et al., 2007).

The requirement to utilize FMD-free cattle to produce sizable lesions following immunization was a drawback of Waldmann's procedure. Prof. Frenkel a Dutch researcher, produced the second advancement in the development of the FMD vaccine. Making use of Maitland's research on tissues preserved in a particular medium (Lombard et al., 2007).

After a healthy cow was killed in a typical abattoir, Professor Frenkel had a wonderful idea to harvest epithelial fragments from the animal's tongue. The tiny fragments of were infected with a potent seed virus and kept for at least 48 hours at 37°C under oxygen bubbling (Lombard et al., 2007).

After the cultural period, the FMD virus was present in both the epithelial cells and the medium. The virus propagated in the epithelial cells. At a meeting done by OIE in Bern in 1947, the procedure was presented as experimental, but commercialization didn't begin until 1950(Lombard et al., 2007). The amount of virus FMD recovered per animal was 1 hundred times greater than with the method done by Waldmann (400 commercial doses), and the animal's immunization status had no impact on the virus's ability to multiply. Saponins were first employed as an adjuvant in the gel of aluminum hydroxide by Espinet in Chile in 1951 (Espinet, 1951).

The employment of the cells, firstly in monolayer and subsequently in a suspension, to meet the enormous requirements for millions of liters of vaccine for immunization campaigns being developed in South America or Europe, was the third important technical turning point in the history of the FMD vaccine production. Monolayer cells were mostly employed industrially in Italy. Initially, primary or secondary kidney cells (from calves, lambs, or piglets) were used, which were obtained from the abattoirs.

The benefits of using a clean cell line, such as the baby hamster kidney cell line (BHK 21) subsequently became clear, quickly outpaced the ability of plants to create vaccines employing cell monolayer growth in roller bottles, and the harvest of many thousands of the bottles wasn't without the risk of bacterial contamination. As a result, producing enormous quantities of vaccines using cell culture in suspension became the preferred technique(Lombard et al., 2007).

The main benefit of this novel technology was that everything can now be done in a very completely closed circuit, including the growth of cells, infection of the cells with sterile seed viruses, clarification of the virus harvesting process, inactivation of the virus, concentration and adjuvant formulation, and, finally, filling of vaccine vials. Following the implementation of effective mass immunization campaigns, FMD outbreaks were uncommon during the 1970s (Lombard et al., 2007).

Because allergens that may come from cell culture were included in and then mixed with vaccine components and caused allergenic reactions during routine vaccination and immunization campaigns, this technique had a distinct but significant drawback. It required ten years to perfect the purification processes thus a non-allergenic and powerful vaccine could be manufactured in large quantities without reducing viral output (Adamowicz et al., 1974).

In areas like South America, where cow breeding was widespread, vaccination of cattle with an oil-adjuvant vaccine had a promising future in the early 1970s. Because they provided a fresh strategy to

address historical shortcomings in disease control, oil-adjuvant vaccinations were well welcomed in that region from both an immunological and political standpoint (Sutmoller & Barteling, 2003). Intramuscular injections of oil-adjuvant vaccinations appeared to produce longer-lasting protection than earlier aqueous vaccines and protected cattle under a wide range of breeding settings (Sutmoller & Barteling, 2003).

1.2.4 Scientific discoveries

The Moosbrugger paper from 1948 had made it widely revealed that FMD vaccinations could still be virulent a few days after their manufacture date even after being inactivated with formaldehyde. The tests for inactivation conducted in the 1950s proved that formaldehyde as inactivate was not a first-order (Moosbrugger, 1948).

Brown and Crick, examined the characteristics of aziridines, a new family of inactivants that was utilized for the first time by Pay et al. in the vaccine industry (Brown & Crick, 1959). But Bahnemann in 1973 made the breakthrough, who showed how a halo ethylamine, typically 2-bromo-ethylamine, can be used by vaccine manufacturers to create an aziridine, the cyclized ethylene-imine, immediately before the inactivation process begins (Bahnemann, 1973).

The procedure was quickly adopted on a global scale and frequently reproduced for biosecurity purposes using a double inactivation process. Since the development of this technique, vaccination doses that may reach hundreds of billions have been examined for their safety globally; not a single one is pathogenic (Lombard et al., 2007).

New studies on the function of non-structural proteins (NSPs) of FMD virus in the immune response also their potential diagnostic use were later conducted in laboratories engaged in FMD research in the middle of the 1990s (Food and Agriculture Organization (FAO), 1998). These results were revolutionary because they enabled vaccination campaigns to employ a vaccine without NSPs without interfering with the ability to identify animals that were virus-infected or virus carriers using serology. All vaccine producers wished for such, as they were the ones receiving the blame for their products' ability to conceal illness risk behind the immunity provided by immunization (Lombard et al., 2007).

Finally, FMD vaccination was feasible with the DIVA (Differentiating Infected from Vaccinated Animals), which was a significant shift with numerous repercussions for the perception and application of the FMD vaccine. For those manufacturers using mainly BHK cells, the removal of heterologous unusual proteins from cell culture due to their bad allergenic properties and of FMD viral NSPs from virus culture due to their interference with the serological tests for diagnosis made antigen purification a dual requirement (Lombard et al., 2007).

This industrial problem was resolved thanks to technical advancements such as chromatography, the usage of polyethylene glycols, and the high polymers of ethylene oxide, which did not compromise the effectiveness of FMD vaccinations. The high level of concentration of viral antigens, from 250 - 1000 times, that resulted from the FMD antigens' thorough purification process proved advantageous (Adamowicz et al., 1974).

1.2.5 Conventional and new-generation vaccines

In this section, we provide a quick overview of the numerous conventional and modern vaccinations on the market and evaluate their benefits, drawbacks, and applicability to the prevention of FMD. A different vaccination or improvements to the ones that are already available are still being sought after(Lu et al., 2022).

The development of successful FMD vaccines using novel methods, including subunit vaccines, plant-based edible vaccines, DNA vaccines, vectored vaccines, vaccinations by virus-like

particles, peptide vaccines, and others, has been made possible by advancements in vaccination (Figure3) (Kamel et al., 2019; Robinson et al., 2016; Shahriari & Habibi-Pirkoohi, 2018). However, they still need to be used successfully as substitutes for the usual whole virus-based vaccine

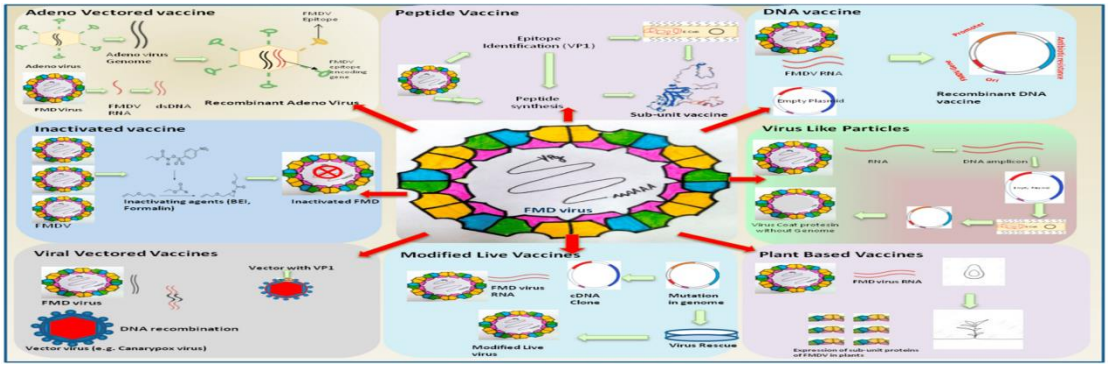

Figure3. The upcoming upgrades in vaccines against FMDV **(Singh et al., 2019)**

1.2.6 Inactivated whole Virus Vaccine

Inactivated viral vaccines were the first utilized and are currently the commercial FMD vaccine. Formalin was first used to eliminate viruses that were still alive in the 1930s. Midway through the 1960s, the FMD formalin-inactivated vaccine, produced in hamster kidney (BHK) cells, significantly decreased the prevalence of the disease in various European nations (Lu et al., 2022). In the 1970s, there were hardly any outbreaks in Europe. After the European Union (EU) forbade immunization against FMD in the 1990s, emergency vaccination in the case of an outbreak was authorized (Leforban & Gerbier, 2002). The most widely used FMD vaccinations worldwide are inactivated versions. The entire virus is often cultivated in suspension culture, chemically inactivated—most commonly with binary ethylenimine (BEI), and combined with the adjuvants (Rodriguez & Gay, 2011).

Formulations based on water, oil, and aluminum are available. Depending on the country's epidemiological situation and the manufacturer, more than one serotype is frequently included in the process of vaccine formulation (Parida, 2009). In vaccination formulations, the antigen payload ranges from three to six PD50. In the event of epidemics in FMD-free areas, highly concentrated vaccinations containing 6PD50 that start working within a week of administration are beneficial. To guarantee effective protection, choosing the right vaccination strain requires rigorous quality control (Commission & Committee, 2008).

For quality control testing, the FAO and the OIE recommend checking for FMDV non-structural proteins as well as identification, sterility, safety, potency, and efficacy. The short period of protection, thermolability of the vaccine, and the requirement for a strictly managed biosafety from level III facility to minimize viral leakage possibility during vaccine manufacture are all drawbacks of using inactivated vaccine formulation (Commission & Committee, 2008).

Additionally, even the purified vaccine formulation occasionally contains minute amounts of those called non-structural proteins (NSPs), causing the development of antibodies specific for NSP and interfering with the DIVA assay (Brocchi et al., 2006; Paton et al., 2006; Robiolo et al., 2006).

Due to the absence of cross-protection across different FMDV serotypes, the most significant problem in manufacturing the inactivated vaccines is the choice of pandemic viral strains. Protection even between specific strains of the same serotype is insufficient (Maradei et al., 2014).

1.2.7. Modified Virus Inactivated Vaccines

To get over the restrictions of inactivated vaccines made with wild-type viruses, namely foolproof DIVA lacking and the requirement for biosafety, especially level III facilities, various organizations have created inactivated vaccines with modified viruses (Bhatt et al., 2018). These vaccines offer DIVA compatibility without requiring non-structural proteins to be purified (Biswal et al., 2015). There is also less need for a high-level biosafety level facility for vaccine production because viruses may culture in the cell culture and also be safe for the target animals (Kamel et al., 2019).

New modified viruses are being created as attenuated vaccines as a result of a better understanding of viral virulence factors (Diaz-San Segundo et al., 2016). Beginning with BEI inactivation and a modified virus that lacks the leader protease coding region also called leaderless, it was demonstrated to offer immunity comparable to the one of the wild type virus. Later, the leaderless virus was further modified by changing the 3D and 3B proteins to enhance DIVA capability (P. Li et al., 2014). In the same way, the 3A protein's deletion of certain residues produced results that were just as successful in pigs but that need to be investigated in cattle (P. Li et al., 2014).

1.2.8. Live Attenuated Vaccines

All live attenuated vaccines (LAVs) have the benefit of inducing long-lasting immunity, which is their main advantage. When LAVs were first developed, they had a high cell culture passage rate; but, because of the likelihood that they might revert to the virulent form, they were not further exploited (Diaz-San Segundo et al., 2016). Because of molecular virology developments, it is now available to change the virulence-related genes, lowering the likelihood that the host will revert (Kamel et al., 2019). Researchers have developed modified new viruses by changing harmful genes, codons, and replication fidelity. Those have shown protection in animals with significantly varying degrees of efficacy (Díaz-San Segundo et al., 2012).

Though there have only been a few investigations in animals, it has been established that FMDV's leader protein can be altered, as can one of the virus' two translation sites of initiation (Jonathan Arzt et al., 2014). It had shown that RNAs that carry the deletion of the stem-loop in the 3′ UTR on the serotype O FMDV genome were harmless when injected into pigs but triggered particular cellular and humoral immune responses (Rodriguez Pulido et al., 2009). Before using FMD live attenuated vaccine there is still considerable work to be done (Lu et al., 2022).

1.2.9. Viral Vector Vaccines

To express structural proteins in those vaccinated animals, several groups delivered FMDV sequences using mammalian viral vectors, such as the poxvirus, herpes virus, and adenoviruses. This resulted in the activation of an efficient immune response against FMDV (Kamel et al., 2019).

The most commonly used viral vector is Vaccinia viruses and human adenoviruses (Berinstein et al., 2000). Initial studies only found limited protection, whereas later changes led to higher-quality vaccines (Gullberg, Polacek, et al., 2013) (Gullberg, Muszynski, et al., 2013). Human adenovirus recombinant-replication defective can be considered one of the most promising vectors for transmitting the FMDV capsid sequence to animals. Pigs and calves that were exposed to the vector of adenovirus bearing the capsid and the 3Cpro coding region were completely protected (de Avila Botton et al., 2006; M P Moraes et al., 2002; Pacheco et al., 2005).

The neutralizing antibody response can be further enhanced in endemic environments by booster injection in cattle. If a vaccine is going to be utilized in endemic environments, this is a crucial and

necessary component. A full-length 2B coding region and increased FMDV capsid protein synthesis are two ways that the vaccine is continuously being improved (Mauro PiresMoraes et al., 2011) or adding RGD motif for better adenovirus transduction in immune dendritic cells (Medina et al., 2016).

One of the most promising approaches of the new generation vaccines involves the delivery of the FMDV capsid via recombinant adenovirus, the monovalent formulation may be used to combat outbreaks of FMD in previously FMD-free areas. However, there is still debate about its use in endemic environments because different virus serotypes produced varying immune responses, which were linked to the differential efficacy of the polyprotein (P1-2A) (Sreenivasa et al., 2017).

1.2.10. Virus-Like Particle Vaccines

Large particles known as virus-like particles (VLPs) are made up of one structural protein or more than one from the virus but lack viral nucleic acid and cannot multiply. They resemble virus particles in terms of their general structure. VLPs are a perfect replacement for the conventional inactivated virus in the manufacture of vaccines because they also have some degrees of safety in addition to maintaining the spatial conformation of the viral natural particles plus epitopes which trigger the creation of neutralizing antibodies (Quattrocchi et al., 2020).

It has been demonstrated that VLPs experimentally excite dendritic cells similarly to inactivated FMDV and also produce humoral immunity (Quattrocchi et al., 2020). VLPs have several benefits, such as improved DIVA capability, decreased biosafety level III facility need, and affordability. VLPs are typically created in a baculovirus-expressing system and then purified (Bhat et al., 2013). In the process of VLPs, Several advancements have been made using the dual promoter vector, the 3Cpro (Ruiz et al., 2014), using the bicistronic complementary DNA cassette that contains 2 open reading frames that encode an FMDV capsid gene called (P1-2A) also 3Cpro that is separated by an internal ribosome entry site (Srinivas et al., 2015).

Another study found that a mutation in the VP2 area made VLPs more thermostable and provided guinea pigs with adequate protection (Ganji et al., 2018). A study using chimeric VLPs that contained the FMDV 3A protein's T cell epitope exhibited a strong immunological response (Crisci et al., 2012). However, it needs to be examined to see if it can be produced and used on a big scale in endemic environments. In other investigations, cattle were successfully immunized with recombinant silkworm baculoviruses encoding the entire P1-2A gene and 3C protease coding areas of serotypes Asia 1 or serotype A of FMD (Z. Li et al., 2012). Eri silkworms have been used to express VLPs of Indian vaccine strains, but no animal testing has been done (Kumar et al., 2016).

VLPs have the potential to be an advantageous replacement for traditional inactivated vaccines, similar to adenovirus vector-mediated delivery. The biggest benefit is the lower price compared to other options. Recently, Xiao et al. (Xiao et al., 2016) also demonstrated the usage of VLPs which are expressed in a prokaryotic system for cattle protection (Xiao et al., 2016). Escherichia coli (E. coli) also is a widespread method of expression for FMDV VLPs. In E. coli, an ideal tandem arrangement (VP0, VP3, VP1) is used to express the capsid protein of the FMDV serotype O, which contains a tiny ubiquitin-like modifier (SUMO) (Xiao et al., 2021).

Assembled FMDV VLPs may expose several epitopes in this manner and are around the same size as the original FMDV. In addition to successfully generating in swine the cellular and humoral immune response that is specific to FMDV, with dose increasing the efficiency had improved. Li et al. changed

specific amino acids to boost the stability of the VLPs, and after that, they screened the modified VLPs for improved VLP yield, higher hydrophobic force within the capsid, and immunogenicity of the VLP vaccination (L. Li et al., 2021).

1.2.12. DNA Vaccines

Theoretically, DNA vaccines provide several benefits, including quick integration of gene sequences from many virus strains or serotypes, improved thermostability, and marker gene incorporation, and most important benefit is the lack of a requirement for manufacture at a facility with biosafety level III.However, several issues need to be resolved before the vaccine is used in the field (Dhama et al.,2008).

Early studies utilizing 3Cpro and DNA encoding the complete capsid showed that several inoculations and a substantial amount of DNA are needed to produce modest levels of neutralizing antibodies (Cedillo-Barrón et al., 2001). Plasmid-delivered genes that encode T and B cell epitopes likewise failed to yield good results (Borrego et al., 2011; Ganges et al., 2011).

The exact explanation couldn't be determined, but efforts were made to include immune system-stimulating proteins in vaccine formulation, such as bovine IL-18 plasmid CDNA and Bcl-xL anti-apoptotic, which improved the outcomes (Kotla et al., 2016). In the same way, an effort was undertaken to enhance DNA vaccines using the purified recombinant FMDV-specific multi-epitope protein called (rMEG990) and also an enhanced sindbis virus replicase-based DNA vaccine expressing that protein; marginally improved outcomes in India were reported (Dar et al., 2013). DNA vaccines have the potential to develop future vaccinations like modified LAVs. DNA vaccines need more time before they may be considered for use(Lu et al., 2022).

1.2.13. Peptide Vaccines

This vaccine collection comprises of group of immunogenic peptides that are either expressed or synthetic in prokaryotic or eukaryotic systems (Wong et al., 2000). A biosafety level III facility is not necessary to generate a formulation of a highly purified vaccine with the appropriate proteins because the handling of live viruses is restricted (Shao et al., 2011).

But like DNA vaccines, peptide vaccinations only provide partial protection and necessitate repeated booster shots (Zhang et al., 2015). However, few papers demonstrate a peptide FMD vaccine's ability to completely protect pigs from the disease (Blanco et al., 2016).

Many antigen delivery strategies, such as using plants infected with a recombinant virus or transgenic plants, have been tested with varying degrees of success (Shao et al., 2011). In China, this vaccination method is currently being used, along with others (Zhang et al., 2015).

1.2.14. Plant Based Recombinant Vaccines

Recombinant vaccines produced on a large scale in plants have been demonstrated to be a potential biotechnological technique. Theoretically, plant-based vaccines have several benefits, including the absence of biosafety level III facility needs, cold storage, and increased production costs (A. G. Shahriari et al., 2016).

These vaccines against different viral infections, including FMD, were developed in plants. Mice were used to test the potency of the structural protein of FMD as VP1, which has been expressed in lucerne (Medicago sativa) (Dus Santos et al., 2002). Similar to this, tobacco (Nicotianatabacum) chloroplasts showed improved expression of VP1 (Z. Li et al., 2008).

However, the technique also faced many additional issues, such as the insufficient protection provided to larger animals and the preprocessing of some leaves before feeding. There weren't many of these

investigations, and they don't seem to be good alternatives to the control program right now(Lu et al., 2022).

1.3 Adjuvants

The commercially available vaccine is normally created using the BHK21 cell culture supernatants of cells infected with the (FMDV), inactivated chemically, partially purified, and then adjuvant-formulated. The inherent risk of this vaccination technology is the discharge of live viruses from the facilities used for production or insufficient inactivation of the virus during vaccine preparation (Parida, 2009).

Therefore, researchers have made an effort to allay these worries by employing new technology to create alternative vaccinations, including genetically modified inactivated vaccines, empty capsid vaccines, and recombinant protein and peptide vaccines (Cao et al., 2013; Guo et al., 2013; Porta et al., 2013; Shao et al., 2011; Uddowla et al., 2012).

Nonliving vaccinal antigens, particularly pure subunit vaccines, are frequently not very immunogenic and also need specific adjuvants to make them immunogenic and give effective protection for a long period (Aguilar & Rodriguez, 2007).

Adjuvants are, in general, chemicals that are used inside vaccine formulation and linked with antigens to strengthen the immune response against them. Additionally, adjuvant use may lessen the number of antigens required or vaccination numbers required to trigger a protective immune response. The non-adjuvanted antigens do not produce certain sorts of immunity (such as Th1 vs. Th2 cells, CD8+ vs. CD4+ T cells, and particular antibody isotypes) as effectively (Coffman et al., 2010).

The ability of several vaccine adjuvants to boost the immune response to FMDV vaccinations has been investigated. These adjuvants include saponins (Quil-A), mineral oil, cytokines, liposomes, Toll-like receptor (TLR) ligands, and others. Without a thorough understanding of their molecular and cellular modes of action, the majority of them have been produced empirically. In this study, adjuvants for FMD vaccines are described in terms of their modes of action and immunostimulatory effects, both historically and today(Cao, 2014).

1.3.1 Mineral oil

The effectiveness of the oil adjuvant is related to the depot building at the site of injection, a means of transporting antigens throughout the lymph system, and antigen release delay with activation of antibody-producing immune cells. The mineral oil-based adjuvant montanide ISA-206 contains anhydromannitol esters in an oily solution and octadecenoic acid. Many Asian and South American nations currently use this adjuvant, which creates a water-in-oil-in-water (w/o/w) emulsion, to create FMD vaccinations(Cao, 2014).

For the FMD vaccine, the double oil emulsion vaccines are now chosen because they are perfect for emergency vaccination and can be utilized to give protection to all susceptible species (Cao, 2014).

Additionally, compared to vaccines with Al (OH) 3 adjuvant, those with oil adjuvant produce stronger and longer-lasting immune responses. Following a single intramuscular vaccination with an FMD vaccine containing an inactivated antigen and oily adjuvanted with Montanide ISA-206, the antibody responses specific for FMD were monitored for at least 92 to 120 days in cattle, 141 days in pigs, and 168 days in sheep. Protective immunity was maintained for at least 218 days in pigs using the oil adjuvant formulation against the disease (Barnett et al., 1996; Barnett & Carabin, 2002; Patil et al., 2002).

Seppic Inc., France (SEPPIC) has recently created the mineral oil-based adjuvant known as Montanide ISA-201. This adjuvant keeps the benefits of ISA-206 while enhancing cellular responses by adding additional chemical components based on ISA-206. Comparatively to ISA-206, inactivated FMD vaccinations with

the ISA-201 adjuvant generated faster and stronger neutralizing antibody responses, as well as greater cellular immunity and protective effectiveness in cattle (Dar et al., 2013).

1.3.2 Saponin based adjuvants

The immunostimulating complexes (ISCOMs) are made up of immunogen, phospholipid, cholesterol, and saponin. ISCOMs have also utilized saponin, such as Quil-A, as a component. The adjuvant activity of the saponin component is retained when it is included in vaccines based on ISCOMs but with less toxicity. ISCOMs have been proven to produce potent helper and cytotoxic T-cell responses as well as high-titer, long-lasting antibodies (Rimmelzwaan et al., 2000; Sambhara et al., 2001).

Dendritic cells (DCs) in draining lymph nodes can take up antigens and retain them for a longer period thanks to ISCOMs, which also cause DCs to become activated and produce potent B and T cell immune responses (Maraskovsky et al., 2009). In addition to enabling significant presentation of MHC class I and inducing both CD4+ and CD8+ T-cell responses to many varieties of soluble protein antigens in both humans and experimental animals, they are powerful enhancers of both Th2 and Th1 cells (Davis et al., 2004).

1.4. Development of FMD vaccine in Egypt

In Egypt, FMD vaccines had many trials and there has been much research on it for many years. Those trials are collected and summarized in the upcoming table 1.

Table (1): Trials of development of FMD vaccine in Egypt since 2005:

Vaccine	strain	adjuvant	Reference
Monovalent FMD vaccine	O1/3/93	Aluminum hydroxide gel (AL (OH)3	**(F.M. et al., 2005)**
Bivalent FMD vaccine	O1 and) A/Egypt 2006)	Aluminum hydroxide gel (AL (OH)3	**Selim et al., 2010))**
Bivalent FMD vaccine	O1 and) A/Egypt 2006)	MontanideIS A 206	**Selim et al., 2010))**
Bivalent FMD vaccine	type O1/Aga/ EGY/93 strain and A/EGY/1/2006	Montanide ISA 206	**(El-sayed et al., 2012)**
Bivalent FMD vaccine	O Manisa and A 22 IRAQ	oil Montanide ISA-206	**(Saad & Deghaidy, 2012)**
Bivalent FMD vaccine	O1 /3/93 EGYPT and Type A EGY /06	oil Montanide ISA-206	**(Saad & Deghaidy, 2012)**
Bivalent FMD vaccine	O1 /3/93 EGYPT and Type A EGY /06	Aluminum hydroxide gel (AL (OH)3	**(Saad & Deghaidy, 2012)**
Bivalent FMD vaccine	O Manisa and A Iran 2005	Aluminum hydroxide gel (AL (OH)3	**(Saad & Deghaidy, 2012)**
Trivalent FMD vaccine	O Panasia-2/A Iran-05/ SAT2 EGY-A-2012	oil Montanide ISA-206	**(El-Bagoury et al., 2015)**
Trivalent FMD vaccine	O Manisa /A Iran-05/ SAT2 EGY-A-2012	oil Montanide ISA-206	**(El-Bagoury et al., 2015)**

Trivalent FMD vaccine	O pan asia, A/Iran 05 and SAT2/EGY/2012	oil Montanide ISA-206	**(Bagoury & Baiomy, 2017)**
Trivalent FMD vaccine	O pan asia, A/Iran 05 and SAT2/EGY/2012	oil Montanide ISA-206	**(Bagoury & Baiomy, 2017)**
Trivalent FMD vaccine	O Pan Asia 2012, A/Iran 05, and SAT2/Egy/2012	oil Montanide ISA-206	**(Hassan et al., 2018)**
Monovalent FMD vaccine	O/PanAsia-2 of the ME-SA topotype	Montanide ISA 50 and saponin	**(A.-H. I. Bazid et al., 2021)**
Heptavalent FMD vaccine	A-Iran05, A-Africa-IV, O-PanAsia2, O-Manisa, O-EA3, SAT-2 Gharbia, and SAT-2 LIB-12	oil-adjuvant (ISA 206)	**(A.-H. Bazid et al., 2023)**

Table1. Illustrates the development of FMD vaccines applied in Egypt since 2005. and it is clear that strain" O" is the most and the main strain that is included in all vaccines. Then in 2010 a new strain "A" was included in the vaccine to become bivalent Afterthat in 2015 another strain added "SAT-2" to become trivalent. In 2023 another topotype was added to the vaccine to finally become a heptavalent one.

CONCLUSION

Inactivated FMD vaccines have many limitations and disadvantages. Live attenuated vaccines still need further studies and investigations. Subunit and VLPs are the promising future FMD vaccines.

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