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Review Article

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DNA Vaccines Against Foot-and-Mouth Disease: A Novel Biotechnological Strategy

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ABSTRACT

This review article points out the Foot-and-Mouth Disease (FMD) as a major global animal disease of serious economic importance and limitations of conventional inactivated whole-virus vaccines. It gives DNA vaccine technology as a potential and safer way of achieving immunity by expressing microbial antigens in host cells and thereby eliciting both humoral and cellular immunity responses. The principles and mechanisms of the DNA vaccines are described in the article: antigen presentation, activation of T- T-cells, as well as the functions of adjuvants, the delivery methods, and electroporation, gene gun, and nanoparticles in the increasing of their efficacy. In addition, it summarizes the advancement in the FMD DNA vaccines against diverse viral proteins such as VP1 and the P1 polyprotein, reviews conducted experimental studies and studies in efficacy trials on animal models and the targeted livestock animal.

INTRODUCTION

Foot-and-mouth disease (FMD) is a paramount animal disease and zoonotic with serious, highly contagious, and economically ruinous viral illness that affects millions of cloven-hoofed animals all over the world (cattle, pigs, deer, goats, and sheep) [1]. Animal disease pathogen, first identified as a virus in the 16th century, and the very first animal disease pathogen identified (Makes FMD a disastrous threat for animal agriculture and animal byproducts) [2]. The disease not only deteriorates the commercial values of livestock by loss of weight and milk output, but also results in enormous economic loss to livestock producers and industry [3]. Involving fever and manifestation of vesicular lesions of the mouth and feet, widespread patterns and rapid transmission of FMD as well as the presence of seven serotypes of the foot-and-mouth disease virus (FMDV) pose challenges to the control [4]. For several decades now, inactivated whole-virus vaccination has been the most helpful approach to the prevention and

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control of FMD. Nevertheless, even though, after more than 70 years, there is a vaccine at our disposal, FMD is an endemic disease in the world. There are a few drawbacks associated with these traditional vaccines, such as a lack of induction of long-term protection, restricted antigen coverage, and a lack of ability to curb infection [5]. Additionally, production of inactivated vaccines requires a high level of bio-containment, and they entail risks of release of live FMDV into the environment. Their short shelf life and the requirement of very strict cold-chain maintenance are other logistical issues of a considerable scale [6]. Among these, the technology of DNA vaccines has risen as one of the most important fields of research and development. This rather novel biotechnological approach that has been discussed for more than three decades in the hope of satisfactory new vaccines has several advantages [7]. DNA vaccines operate on the principle that genetically engineered DNA is used to produce an immunologic response, and this DNA permits the expression of microbial antigen through inside host cells. This may promote antigen presentation via the major histocompatibility complex and stimulate both humoral and cellular immune responses, opening an avenue for a fresh and possibly safer tactic in the war against FMD [8]. The advances in biotechnology have opened new doors for synthesizing specific proteins through microorganisms, and the DNA vaccines were a breakthrough made in that regard [9].

FMD: A Global Threat

FMD is one of the major animal diseases that is linked to a severe and highly contagious, economically devastating viral infection of numerous cloven-hoofed animals [10]. Even though vaccines against FMD have been available for more than 70 years, the disease is still endemic in much of the world and is a constant danger to animal agriculture and international trade. Epidemics may cause significant monetary losses and disrupt the production of animals and animal products. The high rate and varied nature of the FMD virus require intervention measures to avert the destructive effects [11].

Etiology and Pathogenesis of FMD Virus (FMDV)

FMD is caused by the FMDV. FMDV is the prototype of the genus Aphthovirus in the family Picornaviridae [12]. It is a virus that consists of RNA with an icosahedral capsid. FMDV is known for the existence of seven serotypes that are reported to be immunologically distinct: A, O, C, SAT1, 2 & 3, and Asia1 [13]. There is also a great genetic and antigenic variation of strains within each serotype. Historically, the first animal disease pathogen to be found as a virus was FMDV [14]. Nasopharynx is usually where FMDV infection starts in cattle, while in pigs, it is the oropharyngeal tonsils. The virus then rapidly replicates in these habitats before getting disseminated throughout the body through the bloodstream [15]. This viraemic phase results in the emergence of typical vesicular lesions at the mouth, the feet, the snout and the teats on the affected animals. The children of the disease may be higher in young animals with occasional mortality as a result of myocardial degeneration. FMDV is also able to establish a carrier state, especially in cases of cattle, in which persistently infected animals can shed the virus [16].

Transmission and Economic Impact

FMDV can be transmitted through various routes. These include direct contact with infected animals, as well as indirect contact via contaminated materials such as agricultural tools, vehicles, and animal products [17]. Airborne transmission over short distances can also occur. Factors such as animal movement, trade, community grazing, and insufficient surveillance contribute to the spread of the disease [18]. The primary routes of FMDV transmission are seen. Infected animals shed the virus through breath (aerosols), secretions and excretions (contaminating various surfaces), and animal products. This contamination can then lead to new infections through direct contact with aerosols, indirect contact with contaminated materials, or ingestion of contaminated products (Figure 1).

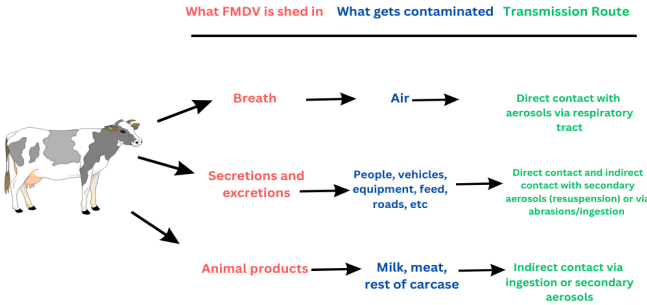

Figure 1: The Pathways of FMDV Spread from Infected Animals Through Breath, Secretions/Excretions, and Animal Products, Leading to Contamination and Subsequent Infection Via Direct or Indirect Contact

FMD poses a major economic threat to agriculture worldwide. Outbreaks result in direct losses due to reduced weight gain, decreased milk production, and mortality, especially in young animals [19]. Affected countries often face trade embargoes, leading to significant financial losses for livestock producers and the industry as a whole [20]. The 1997 outbreak in Taiwan, caused by a CHY topotype virus, resulted in the slaughter of over 4 million pigs and over 6 billion U.S. dollars in financial losses. Control and eradication efforts also incur substantial costs [21]. Vaccination with inactivated whole-virus vaccines has been the most widely used method for prevention and control for over 70 years [22]. In India, a nationwide FMD Control Programme (FMD-CP) using an indigenously produced killed trivalent vaccine has shown encouraging results in reducing disease incidence. However,

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conventional vaccines have limitations such as failure to induce long-term protection, narrow antigenic coverage, and inability to prevent infection [23].

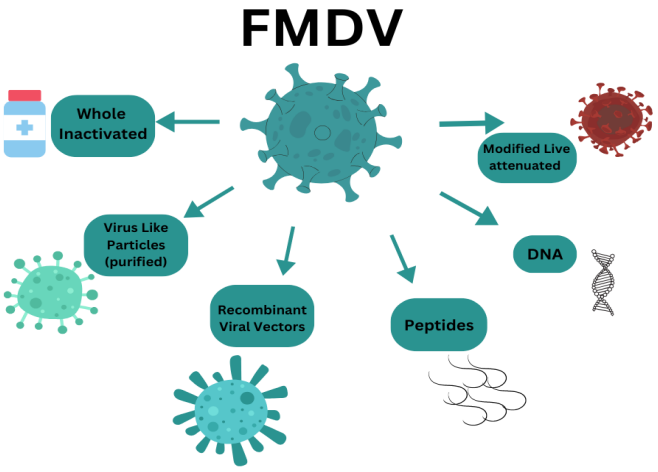

Figure 2: Various Approaches Including Whole Inactivated, Modified Live Attenuated, Virus-Like Particles, Recombinant Viral Vectors, DNA, and Peptides

Delivery Methods (Electroporation, Gene Gun, Nanoparticles)

The efficacy of DNA vaccines can be influenced significantly by the method of delivery, which affects the uptake of plasmid DNA by host cells [47]. Several approaches have been explored to enhance DNA delivery and immunogenicity for FMD vaccines. Electroporation involves the application of brief electrical pulses to the injection site, which transiently increases cell membrane permeability, facilitating DNA uptake [48]. This method increases the transfection efficiency of DNA vaccines in vivo, provoking a better immune response to FMDV. Gene gun (biolistics) involves a mortar-like tool to shoot the gold micro particles coated with DNA right into the skin with the help of the gas pressure [49]. This approach attacks the antigen-presenting cells in the skin and has been used for FMD DNA vaccination to show the development of the immune responses. Nanoparticles like mannosylated chitosan nanoparticles and calcium phosphate nanoparticles have been examined for use as the delivery method for the FMDV DNA vaccines [50]. These nanoparticles can prevent the degradation of DNA and can increase cellular uptake and improve the immunological values and protection for viral challenge. For instance, mannosylated chitosan nanoparticles loaded with FMDV VP1-Ompa DNA vaccine indicated a promising immunological evaluation in guinea pigs [51].

Immunological Effects Caused by FMD DNA Vaccines

The DNA vaccines for FMD target both humoral and cellular immune responses with the delivery of genetic materials encoding the antigens of FMDV into host's cells. These vaccines utilize host's cells for production of viral proteins that are processed and presented to the immune response [52].

Humoral and Cellular Immunity

FMD DNA vaccines can produce neutralizing antibody responses, which are known to be the primary immunity against FMDV. The major capsid protein VP1 protein that is frequently targeted to induce these antibodies. For instance, a DNA construct of a chimeric core-VP1 virus-like particle caused far greater amounts of antigen-specific IgG production and neutralizing antibodies in mice than a regular VP1 DNA construct [53]. Other than humoral immunity, cell-mediated immunity (CMI) can be stimulated by FMD DNA vaccines [54]. The endogenously expressed antigens are presented via both MHC class I and class II pathways, leading to the activation of these T cell subsets. Studies have shown that FMD DNA vaccines can induce FMDV-specific T cell proliferation and CTL responses [55].

T-Cell Activation and Memory Response

Upon uptake of the DNA vaccine by host cells, including antigen-presenting cells (APCs) like dendritic cells, the encoded antigen is expressed and processed [56, 57]. FMD DNA vaccination can lead to the generation of memory T cells, contributing to long-term immunity [58].

Role of Adjuvants and Co-Stimulatory Molecules

The immunogenicity of FMD DNA vaccines can be significantly enhanced by the use of adjuvants and co-stimulatory molecules. Due to the sometimes lower immunogenicity of DNA vaccines compared to traditional vaccines, various strategies have been employed to boost the immune response [59]. Genetic adjuvants, such as cytokines and chemokines, can be co-expressed from the same plasmid or delivered separately. Several cytokines have shown promise in enhancing FMD DNA vaccine efficacy. IL-6 has been shown to advance the cellular immune response and promote the maturation of dendritic cells [60]. IL-15 has been shown to enhance cellular and mucosal immune responses and the level of IFN-γ induced by FMD DNA vaccines [61]. Intranasal administration of FMDV DNA vaccine with IL-15 as an adjuvant induced enhanced CMI. IL-18 can increase the immunogenicity of DNA vaccines. Co-administration of bovine IL-18 with a DNA vaccine gave a protective immune response in cattle. GM-CSF as an adjuvant with a DNA vaccine encoding P1-2A induced robust FMDV-specific and neutralizing antibodies in swine [62, 63]. Delivery methods can also act as adjuvants by improving DNA uptake and transfection efficiency. Electroporation and gene gun delivery have been shown to enhance immune responses against FMDV DNA vaccines. Nanoparticles, such as mannosylated chitosan nanoparticles, have demonstrated substantial

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improvements in immunological parameters [64]. Cationic PLGA micro particles used to coat DNA vaccines have also resulted in long-term immune responses.

Experimental Studies and Efficacy Trials

Experimental studies and efficacy trials are crucial steps in the development of foot-and-mouth disease (FMD) vaccines to evaluate their immunogenicity and protective potential before widespread use. These studies involve rigorous testing in animal models, followed by trials in target livestock species.

Experimental Studies

Mice and guinea pigs are often used as initial small animal models to assess the immunogenicity of FMD vaccine candidates, including DNA vaccines, subunit vaccines, and empty capsid vaccines [65]. For instance, mice have been used to analyze the immune response induced by DNA constructs encoding FMDV antigens. Guinea pigs have been used to evaluate the immunological parameters of nanoparticle-based DNA vaccines. However, it's important to note that results in these models may not always directly correlate with those in natural hosts like cattle and swine. These studies typically measure humoral immune responses, such as the induction of neutralizing antibodies [66]. Assays like plaque-reduction assays are used to detect specific neutralizing antibodies against FMDV in serum samples. Cellular immune responses, including T cell proliferation, cytotoxic T lymphocyte (CTL) responses, and cytokine production (e.g., IFN-γ), are also evaluated [67]. For example, one study compared the immune responses induced by a chimeric core-VP1 DNA vaccine and a regular VP1 DNA construct in mice by assessing IgG production, T cell proliferation, CTL response, and cytokine production. Before in vivo testing, studies might involve in vitro experiments to assess antigen expression in transfected cells. For example, the expression levels of DNA vaccine constructs were studied in HeLa cells and HEK and CHO cells [68].

Efficacy Trials

Efficacy trials in cattle and swine, the natural hosts of FMDV, assess a vaccine's protective ability against viral challenge. Serum neutralizing antibody levels often correlate with protection [69]. Vaccinated animals are challenged with live FMDV, and protection is evaluated by monitoring clinical signs (e.g., vesicular lesions) and sometimes sub-clinical infection and viral persistence [70]. Achieving sterile immunity, preventing both disease and infection/shedding, is a key goal. Trials may assess for viral RNA or non-structural proteins (NSPs) post-challenge; the absence of anti-NSP antibodies (e.g., against 3ABC) often indicates protection from infection [71]. Duration of immunity is also evaluated through challenges at different time points post-vaccination [72]. Efficacy trials have tested novel platforms like DNA, adenovirus-vectored, and recombinant empty capsid vaccines, demonstrating promising results (e.g., adenovirus-vectored A24 subunit vaccine in cattle [73], DNA vaccines in swine). Overall, efficacy testing progresses from small animal models to target species, rigorously evaluating clinical protection and ideally, sterile immunity [74]. Measuring humoral and cellular responses, alongside clinical protection and infection prevention, are critical, as is considering scalability for industrial production [75].

CONCLUSION

In conclusion, this review article highlights FMD as a significant global threat to animal agriculture, underscoring the limitations of traditional inactivated whole-virus vaccines despite their long history of use. It presents DNA vaccine technology as a novel and promising biotechnological strategy that offers several advantages, including enhanced safety profiles and the potential to induce both humoral and cellular immune responses by expressing microbial antigens within host cells. The article details the principles, mechanisms of action, and various approaches to enhance the efficacy of FMD DNA vaccines, such as targeting specific viral proteins like VP1 and the P1 polyprotein, utilising adjuvants and advanced delivery methods like electroporation and nanoparticles. While acknowledging the progress made in experimental studies and efficacy trials, the review also recognises the challenges and limitations that currently hinder the widespread application of DNA vaccines against FMD, paving the way for future research and development efforts to overcome these obstacles and realise the full potential of this innovative approach.

Authors Contribution

Conceptualization: SK, TA
Methodology: BA, EK
Formal analysis: AN, MFT
Writing review and editing: AS, AR, SA

All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

All the authors declare no conflict of interest.

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