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Review Article

Developing Vaccines Against Foot-and-Mouth Disease: a Biotechnological Approach

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ABSTRACT

Foot-and-mouth disease (FMD) is a contagious viral disease of livestock with significant economic effect. It is prevalent in various regions of Asia, Africa, and South America. The causative agent of this disease is called foot-and-mouth disease virus (FMDV), which is a member of Aphthovirus genus. Vaccination is an effective technique to prevent the complications of FMD and to eradicate the disease in contaminated regions. Attempts are being made since the 1930s to develop potent vaccines against FMD. The history of vaccination against FMD has documented various types of vaccines including inactivated viruses and empty capsids, as well as attenuated and recently developed recombinant vaccines. Although the available inactivated virus vaccines effectively prevent FMD, they have several limitations such as expensiveness, short shelf life, and short-lived protection. Therefore, it is essential to provide other types of vaccine. To reach this goal, researchers used various platforms including bacterial hosts, yeast expression system, and mammalian cell culture, as well as microalgae and higher plants to produce recombinant vaccines against FMDV. Green plants offer numerous benefits including low cost, correctly folded recombinant, and improved glycosylation patterns. This study aimed to provide a review of the current status and recent progress in the field of producing effective vaccines against FMDV entailing empty capsid, attenuated vaccines, and recombinant subunit vaccines. In addition, the advantages and disadvantages of each type are described, and the biotechnological improvements of the production of anti-FMD vaccines in plant systems are discussed with prominent examples, thereby confirming the feasibility of plant species as effective bioreactors for the production of recombinant vaccines. To the best of our knowledge, traditional approaches are still the preferred methods to protect livestock against FMD. Modern approaches such as recombinant vaccine production are quite promising. However, they have to pass research and development phase and further trials before they can be registered and launched onto the relevant market.

Keywords: Foot-and-mouth disease, Vaccines, Subunit, Plants

Les vaccins contre la fièvre aphteuse (FMD): une approche biotechnologique

Résumé: La fièvre aphteuse (FA) est une maladie virale contagieuse des bovins et des ovins. Cette maladie entraîne annuellement des pertes économiques énormes pour les unités productrices de viande et de lait. Cette maladie est répandue dans diverses régions d'Asie, d'Afrique et d'Amérique du Sud. Le virus de la fièvre aphteuse (VFA) appartenant au genre Aphthovirus est à l'origine de cette infection. La vaccination est une approche efficace pour réduire les effets nocifs de la fièvre aphteuse et éradiquer la maladie des régions contaminées. Depuis les années 1930, le développement de vaccins efficaces contre cette maladie fut l'objet de nombreuses études. La longue histoire de la lutte contre la fièvre aphteuse a été rythmée par divers types de vaccins produits à partir de virus inactivés, de capsides vides, de virus atténués et de vaccins recombinants plus récemment mis au point. Les vaccins inactivés actuellement disponibles se sont avérés efficaces pour la prévention de la fièvre aphteuse, mais présentent certaines limites telles qu'un coût de production élevé, une courte durée de conservation et une courte durée d'immunité. Ces restrictions rendent le développement d'autres

une confirmation et un repliement précis de l'antigène recombinant, et des modèles de glycosylation appropriés pour la production de vaccins recombinants anti-FA. Dans cet article, nous passons en revue l'état actuel et les progrès récents dans le domaine de la production de vaccins efficaces contre la fièvre aphteuse, notamment les vaccins à base de capsides vides, les vaccins vivants atténués et, plus important encore, les vaccins recombinants sous-unitaires. Les avantages et les inconvénients de chaque type de vaccin sont décrits. Les améliorations biotechnologiques de la production de vaccins anti-aphteux dans les systèmes végétaux sont développées à l'aide d'exemples frappants, confirmant ainsi la pertinence de l'utilisation des espèces végétales en tant que bioréacteurs pour la production de vaccins recombinants. Cette revue de la littérature montre plus globalement que les approches traditionnelles restent encore des méthodes de choix pour la vaccination du bétail contre la fièvre aphteuse. Les approches modernes telles que la production de vaccins recombinants sont très prometteuses, mais sont encore en phase de «recherche et développement» et doivent faire l'objet d'essais supplémentaires avant d'entrer sur le marché. Cet article se termine par une conclusion générale sur les différents types de vaccins contre la fièvre aphteuse.

Mots-clés: Fièvre Aphteuse; Vaccins, Sous-Unité; Les Plantes

INTRODUCTION

Foot-and-mouth disease (FMD) is a major animal disease that affects milk and meat production (Wigdorovitz et al., 1999a). Farmers suffer substantial economic losses from this disease. Devastating effects and quick spread of the disease among cattle and sheep neutralize any effective treatment. FMD occurs throughout much of the world, and its wide host range and fast is a major challenge. FMD is a severe and highly contagious disease affecting the domestic ruminants. Contaminated animals, agriculture tools, and vehicles can contribute to the dissemination of FMD virus (FMDV). Therefore, the development of new effective vaccines is of paramount importance (Rodriguez and Grubman, 2009). FMD is characterized by fever lasting about one week and lesions in the mouths and feet that may result in lameness (Habibi-Pirkoohi and Mohkami, 2015). Cattle, sheep, goats, pigs, and deer are likely to be the main hosts. In addition, several reports demonstrated the infection in hedgehogs and elephants. Other animals such as llamas are resistant to FMD and play a minor role in transmitting the virus. Although rats and chicken are not natural hosts of the virus, they can be infected with FMDV during laboratory experiments. According to the literature, humans are very rarely infected by this virus (Rodriguez and Grubman, 2009). FMD causes a high mortality rate in infected animals. FMDV is a picornavirus, the prototypical member of the Aphthovirus genus (Habibi-Pirkoohi et al., 2014). The disease causes infectious lesions in the mouth and feet of artiodactyls, which pose a number of critical problems to animal husbandry (Li et al., 2008). The virus particle (25-30 nm) is composed of an icosahedral capsid, made of protein, containing a single strand of ribonucleic acid (RNA) (Figure 1).

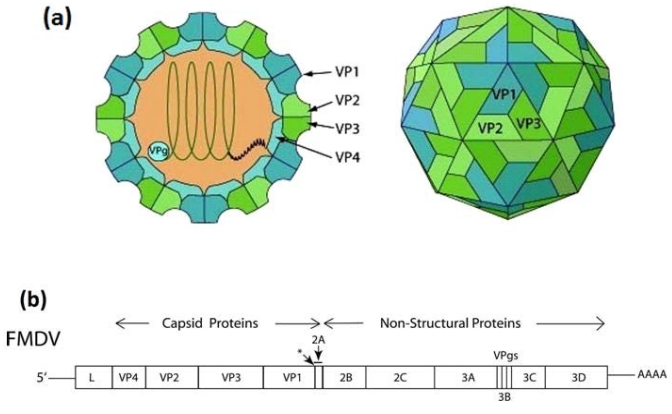

Figure 1. Schematic representation of FMDV. (a): Icosahedral capsid structure, (b): Composition of foot-and-mouth disease virus capsid protein. The capsid consists of both structural and non-structural proteins.

After initial encounter between the virus and the host cell, FMDV binds to a receptor and enters the target cell. Thereafter, the capsid dissolves and the RNA gets replicated and translated into viral proteins using the

translation machinery of the host cell using a cap-independent manner driven by the internal ribosome entry site element (Moraes et al., 2002). A large number of conserved sequences have been identified in the FMDV genome that are crucial for the replication of RNA virus. These sequences can be used to develop a new generation of FMD vaccines (Liu et al., 2017). The symptoms of FMDV infection include the occurrence of blisters in the animal's mouth and feet, the quick disseminating nature, and the numerous virus antigenic types and subtypes (Grubman and Baxt, 2004). Inside the host cell, FMDV first replicates in the pharynx and then invades the blood stream, thus causing scolds in the mouth and feet of the host (Pacheco et al., 2010). Seven distinct serotypes of FMDV have been identified, all of which possess a single-stranded RNA genome and an icosahedral structure. These serotypes include Southern African Territories (SAT1, 2, and 3), Asia1, C, O, and A (Habibi-Pirkoohi et al., 2014). The SAT serotypes are restricted to Africa, while the others are predominant in Asia, Europe, and South America (Figure 2).

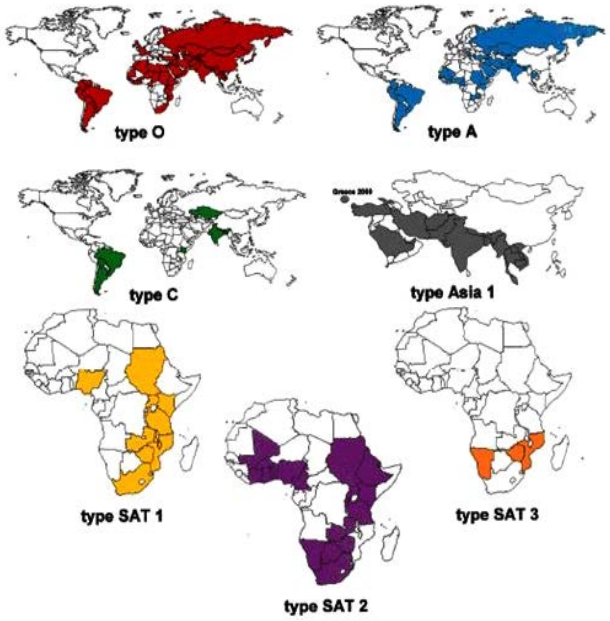

Figure 2. Global distribution of foot-and-mouth disease virus serotypes.

Vaccination is an effective and the only available approach to prevent FMD. There is a global consensus on the fact that vaccination is the most reliable strategy to combat the disease (Habibi-Pirkoohi et al., 2014). The available anti-FMDV vaccines are primarily based on inactivated virus preparations. Although this method is effective, their production is expensive and even risky because the manipulation of massive amounts of virus could result in disease dissemination (Rodriguez and Grubman, 2009). Another problem with current vaccines lies in the fact that the causative agent of FMD is highly unstable. The instability of some serotypes negatively affect the quality of vaccines and the duration of immunity. Moreover, these vaccines should be supplemented with an adjuvant (often oil-in-water emulsion) to induce effective immunity, which increases vaccine manufacturing costs and may be associated with several adverse effects (Park et al., 2016). This study aimed to review the advantages and disadvantages of currently available inactivated vaccines and novel molecular vaccines produced for controlling and eradication of FMD. Particularly, the progress toward the development of plant-based recombinant anti-FMDV vaccines is highlighted. Regarding the fact that various strategies were implemented to develop these vaccines, we have tried to classify different types of anti-FMDV vaccines. The approaches used to develop such vaccines can be classified into transgenic and non-transgenic categories.

MATERIALS AND METHODS

This qualitative study was conducted to assess the history and current status of anti-FMDV vaccines. To reach this goal, a library-based approach was made to write a good literature review about FMD and various types of vaccine developed to combat this animal disease. To the best of our knowledge, the majority of previous studies were carried out into conventional vaccines, and there is a gap between traditional vaccines and modern approaches that are based on recombinant DNA technology. Therefore, this study

was performed to investigate the large number of studies related to conventional and novel anti-FMDV vaccines. Data were collected from the articles about this topic which have been published since early 1970's up to now. Search strategies were selected by content analysis of relevant literature and used as entries for searching reliable scientific databases such as Elsevier, Academic Search, BioOne, Europe PubMed Central, etc.

Inactivated Vaccines. Loeffler and Frosch in 1897 introduced FMDV as the etiologic agent of FMD and anti-FMDV vaccines were presented as an early example of vaccines developed to immunize animals (Martin and Edwards, 1965). The first anti-FMDV inactivated vaccine was developed during 1930s using vesicular fluid obtained from infected animals. Inactivation of the virus was performed by formaldehyde; however, the commercialization of inactivated vaccines was launched two decades later (Zhang et al., 2011). The production of anti-FMDV inactivated vaccine was improved by the growth of FMDV in BHK cell suspension, using ethylene imines for FMDV antigen inactivation, and using oil adjuvants (Rodriguez and Grubman, 2009).

Empty Capsids. Another approach is the use of immunogens that contain the entire repertoire of immunogenic sites of the virus that decreases the chance of the selection of antigenic variants from the quasispecies. Empty capsids are viral particles that are devoid of nucleic acids produced in host cells serving as immunogenic as virulent particles (Grubman and Baxt, 2004). Empty capsids are produced by various systems and assembled into cell culture. Moreover, a wide range of approaches have been adopted to deliver the products. So far, the most effective approach has been the transfer of the sequence of the FMDV capsid with a replication-defective human adenovirus type 5 (Ad5). In a pioneer work, an Ad5 vector containing the capsid sequence of FMDV was constructed and administered to pigs. The animals immunized with a single dose of the vector were protected against the virus challenge (Moraes et al., 2002). Recently, various systems with different properties have been used to express the FMDV capsid along with various cytokines as complementary adjuvants (Li et al., 2008). Some of these studies are highly promising; nevertheless, they are still at the early steps of research and development and further improvements are required.

Attenuated Vaccines. The development of live attenuated anti-FMDV vaccines demonstrated a limited success owing to various pathogenic species or inefficacy of the vaccine. Live attenuated vaccines are mainly based on the selection of attenuated viruses grown in cell cultures or laboratory animals with attenuated phenotypes. Nonetheless, the mechanism of attenuation remained unknown, viral attenuation was incomplete, and immunogenicity was not as effective as that of other types of vaccines. As a result, live attenuated anti-FMDV vaccines have not been applied for many years. However, increased knowledge of the genomic structure of the virus provided great contribution to the production of live attenuated anti-FMDV vaccines (Zhang et al., 2011). The identification of the fact that leader protein of FMDV plays a crucial role in its virulence was an important step toward the improvement of attenuated vaccines. There is still a research gap in the literature about other probable virulence determinants located on the viral genome. The identification of these determinants can lead to the development of novel attenuated.

The Era of Subunit Vaccines. During late 1960s, the analysis of the structure of the FMDV capsid indicated that one of the capsid proteins called VP1 is predominantly exposed (Laporte, 1969). This prominent discovery motivated a large number of scientific groups to use various strategies to develop novel protein-based vaccines as alternatives to the inactivated vaccines. The VP1 peptide was successfully purified from FMDV and induced protective immune response in pigs (Bachrach et al., 1975). By the application of genetic engineering techniques, it was shown that VP1 recombinant peptide expressed in Escherichia coli could protect cattle and pigs against artificial challenges (Kleid et al., 1981). According to

the literature, VP1 could induce neutralization; nevertheless, the genome sequencing of the FMDV revealed a significant difference between different species in terms of VP1 sequence. Synthetic VP1 protein was chemically produced, and its effectiveness in inducing immune response in cattle and swine was confirmed (Bittle et al., 1982). Several studies demonstrated that the combination of VP1 peptide and T cell epitope peptides was recognized by a large number of laboratory animals. Other systems including transgenic plants, yeasts, fungi, transformed plants, viral vectors, and naked DNA were investigated for the production of B cell and T cell peptides (Walmsley and Arntzen, 2000). Generally, different studies recommended that those peptide vaccines which contain only a few epitopes are not effective enough to induce significant immunity. Peptide vaccine should necessarily include VP1 epitopes in combination with T cell and B cell to induce strong immunity. The region of VP1 amino acids 134-138, which is called GH loop, is a strong immunogenic site for inducing immune response. However, GH loop lacks T helper epitopes; therefore, they might not be recognized by major histocompatibility complex molecules and B cells for inducing high affinity neutralizing antibodies. Accordingly, the immunogenicity of GH loop and its feasibility as an effective vaccine decreased (Habibi-Pirkoohi et al., 2014). It was indicated that a peptide corresponding to entire GH loop and adjacent sequences (129-169) could induce immunity in treated animals by applying a peptide-based assay. This peptide includes both T and B cells sites, which enhance immunogenicity of the GH loop. Moreover, this synthetic peptide had a consensus sequence to confront the hypervariability of serotype O viruses (Wang et al., 2002).

Plant-based Recombinant Vaccines. Multiple promising studies were carried out into subunit vaccine production in plant systems by the development of transgenic lines to produce antigens that are capable of inducing strong immune systems in the tested animals (Habibi-Pirkoohi et al., 2014). The application of higher plants as green factories for the production of recombinant vaccines was considered as a promising biotechnological tool for large scale production of anti-FMDV vaccines (Shahriari et al., 2016). A considerable number of experiments were performed to produce plant-based recombinant vaccines against various zoonotic diseases. This technology is based on the expression of a specific antigen in plant host and the application of the recombinant antigen as a potential vaccine against a large variety of bacterial and viral pathogens (Figure 3).

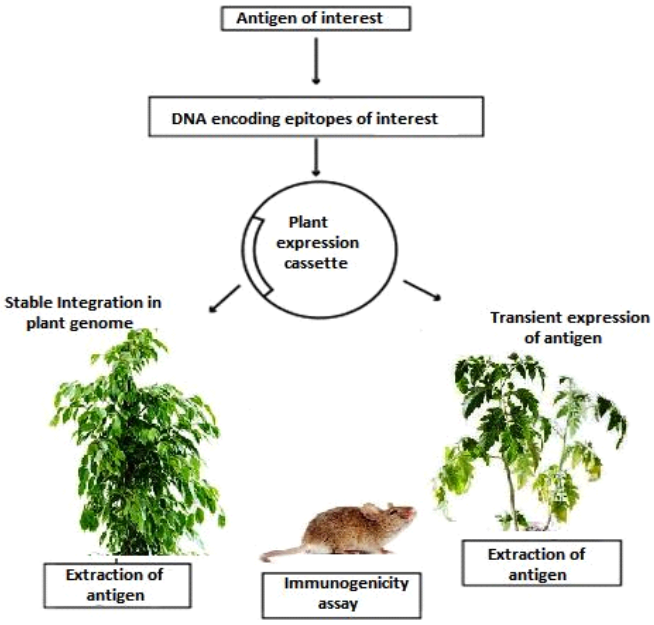

Figure 3. Schematic presentation of the process of producing recombinant vaccines in green plants

The ease of plant genetic manipulation, availability of optimized protocols, and safe nature of green plants have made the technology a promising tool for controlling or even eradication of many diseases (Shahriari et al., 2016). Cell walls of plants protect the recombinant epitopes against digestion in gut. Therefore, this technique is a safe and effective way for the delivery of recombinant vaccine to blood circulation. Moreover, plants are not host for any human or animal pathogens. Transgenic plants can be

stored in room temperature, which lead to diminished cost of storage and transportation (Habibi-Pirkoohi et al., 2014, Habibi-Pirkoohi et al., 2015). The costs for production of transgenic plants are as low as for other agricultural crops, making them an attractive platform for the production of recombinant vaccines (Carter Iii and Langridge, 2002). Considering the efficacy of plant hosts for production of recombinant vaccines and the commercial importance of FMD, it is not surprising to see that many investigations have been carried out to express recombinant vaccines against this disease (Dus Santos et al., 2005). As mentioned above, the most determinant epitopes of FMDV reside on VP1 protein; therefore, this protein is used as the central part in many projects aimed to produce anti-FMDV recombinant vaccines (Table 1). Wigdorovitz et al. (1999b) used the transgenic plants of alfalfa (Medicago sativa) expressing the structural protein VP1 to develop recombinant oral vaccines in mice. Consistent with the results of Dus Santos et al. (2005), Wigdorovitz et al. (1999b) indicated that this recombinant plant-based vaccine successfully induced immunity in the animal model. An epitope-based peptide vaccine spanning amino acids from 135 to 160 of VP1 in alfalfa was previously expressed (Dus Santos et al., 2002). He et al. (2007) developed VP1 protein conjugated with β-subunit of cholera toxin in transgenic potato (Solanum tuberosum) and reported that the recombinant protein can be produced up to 0.13% of total soluble protein (TSP). In addition, VP1 was expressed in the model plant of Arabidopsis thaliana. In a prominent work, Li et al. (2008) expressed VP1 protein in the chloroplasts of tobacco (Nicotiana tabacum) and achieved high level of transgene expression in the host plants. In this study, tobacco plants were transformed by a chloroplast expression vector called pTRVP1 that harbors VP1 and aadA genes as selective markers using micro-particle bombardment method. According to the results of enzyme-linked immunosorbent assay, the transgenic line accumulated the recombinant antigen as much as 3% of TSP. Based on these results, it was stated that by the application of suitable strategy, transgenic plants can be a valuable source for the production of recombinant vaccines against various animal diseases (Li et al., 2008).

Table1. Examples of investigations carried out to develop subunit anti-FMDV vaccines

Gene construct	Strategy	Host	Ref
VP1	Whole *VP1*	*Escherichia coli*	Kleid *et al.*, 1981
VP1	Inclusion of LE** gene	*Escherichia coli*	Morgan and Moore, 1990
VP1	Whole *VP1*	*Arabidopsis thaliana*	Carillo *et al.*, 1998
VP1	Whole *VP1*	*Medicago sativa*	Wigdorovitz *et al.*, 1999b
VP1	Transient expression using TMV vector	*Medicago sativa*	Wigdorovitz *et al.*, 1999a
VP1-135-160	Inclusion of GUS*** for quick identification	*Medicago sativa*	Dus Santos *et al.*, 2002
VP1	*Vaccinia* virus as expression vector	Mammalian cell culture	Brinstein *et al.*, 2000
VP1	Conjugated with IFN-γ*	*Escherichia coli*	Shi et al., 2006
VP1	Specific promoter	*Arabidopsis thaliana*	Pan *et al.*,2011
VP1-129-169	Transient expression	*Nicotiana tabacum*	Hbibi-Pirkoohi *et al.*, 2014
VP1-129-169	Transient expression	*Spinacia oleracea*	Hbibi-Pirkoohi *et al.*, 2015
P12A-3C	Inclusion of Kozak sequence	*Solanum lycopersicum*	Pan *et al.*,2011
VP1	Chloroplast engineering	*Chlamydomonas reinhardtii*	Sun et al., 2003
VP1-129-169	*Agrobacterium*-mediated transformation	*Chlamydomonas reinhardtii*	Hbibi-Pirkoohi *et al.*, 2014
VP1+3D	Codon optimization	*Escherichia coli*	Bae *et al.*, 2009
VP1 epitopes	Tandem repeats	*Escherichia coli*	Shao *et al.*, 2011

* Interferon, ** Leader sequence in tryptophan operon, *** β-Glucuronidase reporter gene

These results are related to the stable integration of foreign genes into plant species to obtain transgenic lines. Nevertheless, recombinant vaccines can be manufactured by transient expression assays (Figure 3).

Wigdorovitz et al. (1999a) expressed VP1 protein in alfalfa leaves using Tobacco mosaic virus (TMV) as expression vector. On the other hand, Habibi-Pirkoohi et al. (2014b) applied Agrobacterium-mediated transient gene expression system to produce an epitope-based anti-FMDV vaccine in tobacco (Habibi-Pirkoohi et al., 2014a), spinach (Spinacia oleracea), and alfalfa (Habibi-Pirkoohi et al., 2014a). Habibi-Pirkoohi et al. in 2014b used agroinfiltration to induce the expression of 129-169 amino acids of VP1 protein in tobacco leaves. Transformation efficiency was confirmed by polymerase chain reaction (PCR) analysis. The expression of foreign gene was confirmed at both transcription and translation levels. Real time PCR revealed the antigen transcription in the infiltrated leaves. Based on ELISA results, high levels of antigen expression were observed in the transformed leaves. Additionally, similar results were obtained with spinach. In addition to terrestrial plants that are extensively used for producing recombinant vaccines, microalgae have gained much attention during recent years as an ideal expression platform (Habibi-Pirkoohi et al., 2014a). The first algae-based anti-FMDV vaccine was a chimeric molecule comprising VP1 and the CTB antigen. The chimeric antigen was expressed in the chloroplasts of Chlamydomonas reinhardtii and reached the concentration of 3-4% of TSP (Sun et al., 2003). Habibi-Pirkoohi et al. (2014a) indicated the feasibility of Agrobacterium tumefaciens for nuclear transformation of microalgae and expressed an epitope-based recombinant vaccine including amino acids 129-169 in nuclear genome of Chlamydomonas reinhardtii. Considering numerous advantages of microalgae, it is expected that these organisms may be used as a permanent host for the production of recombinant subunit vaccines including that of anti-FMDVs. Although the heterologous expression of viral epitopes in green plants is a feasible approach compared to other techniques used for the production of recombinant vaccines, this technology suffers from major drawbacks such as the low expression level of foreign protein in plant tissues. Several strategies including the application of signal peptides, codon optimization, and inclusion of a leader sequence are used to increase the levels of expression of different FMDV recombinant proteins in transgenic plants (Sala et al., 2003). An ideal recombinant vaccine should satisfy the demands of FMD-free countries and eradicate the FMD outbreaks around the world. A general comparison of various anti-FMDV vaccines is presented in Table 2. According to the Table 2, virus inactivation is the preferred approach for vaccination against FMD. This approach has a rich background, and there are well-known procedures that facilitate mass production of

Table2. Advantages and disadvantages of various types of anti-FMD vaccines

Vaccine type	Advantages	Disadvantages	Status	Related references
Inactivated virus	Mass production, high immunizing effect, well established production procedure	Risk of incomplete inactivation, escape of live pathogenic viruses, high production cost	Currently available	Zhang et al., 2011; Rodriguez and Grubman, 2009
Empty capsid	Safety	High production cost	R&D**	Gullnerg et al, 2016
Attenuated virus	Feasibility of mass production, high immunizing effect	High production cost, vulnerable to instability of FMDV* serotypes	Available	Zhang et al., 2011
Recombinant vaccines (non-plant platforms)	Oral administration, lack of virus escape risk	High production cost, need for expensive culture media and laboratory devices, digestion in the gut	R&D	Walmsley et al., 2000; Habibi-Pirkoohi et al., 2014a
Plant-based recombinant vaccines	Oral administration, cost-effectiveness, no need for cold temperature, mass production	Scarcity of field trials; need for purification in some cases (tobacco for example)	R&D	Habibi-Pirkoohi et al., 2016; Shahriari et al., 2016; Li et al., 2006

* Foot-and-mouth disease virus, **Research and development

inactivated virus vaccines. Empty capsid vaccines showed promising results in laboratory experiments; nonetheless, they need to pass several trials to ensure their efficacy. The attenuated vaccines are currently available in the market. However, the instability of FMDV serotypes is a major challenge for this type of vaccination (Zhang et al., 2011). Recombinant vaccines offer several advantages relating to livestock vaccination against FMD. However, high production cost is a major challenge for the commercialization of recombinant vaccines produced in the laboratory. This cost encompasses the cost of media preparation, purification of the expressed antigens, expensive laboratory devices, etc. On the other hand, plant-based recombinant vaccines are fairly inexpensive and can be administered orally. However, such vaccines are still in the primary stage and need much more trials to confirm their immunization efficacy.

Conclusion

FMD is a common and vulnerable disease. Vaccines can be used to prevent the disease; however, they do not provide any guarantee that animals would be prevented from being infected. Currently available vaccines are inactivated viruses, and their effectiveness in immunization is confirmed. Nevertheless, several serious shortcomings of such vaccines have forced researchers to pursue alternative methods for producing vaccines. Many improvements have been made in the vaccine production process. Different types of vaccine including empty capsid vaccine, live attenuated vaccines, and subunit vaccines are currently available. Each vaccine type possesses its own advantages and disadvantages. The production of recombinant anti-FMDV vaccines, especially in plant hosts, is a promising way for safe and cost-effective production of effective vaccines. Large varieties of plant species including microalgae have been implemented as production platforms for recombinant anti-FMDV vaccines. However, there have been many limitations that restrict the commercialization of plant-based recombinant vaccines. Obviously, enhancing the expression level is a key factor that influences the fate of recombinant vaccine production in living organisms. Further studies are recommended to implement gene expression enhancing factors, identify immunogenic sites that are strongly effective in inducing immunogenic site, and evaluate new compounds as vaccine adjuvants.

Ethics

I hereby declare all ethical standards have been respected in preparation of the submitted article.

Conflict of Interest

The authors declare that they have no conflict of interest.

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