

Innovations and Principles of Vaccine Development for Livestock

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1.Introduction

Numerous animal illnesses can be effectively prevented by vaccinations. Numerous successful vaccines developed in the field of vaccination have greatly lessened the impact of a number of serious illnesses in livestock and companion animals. Nowadays, cell membrane chemicals, toxoids, or live attenuated, dead, or inactivated microorganisms make up the great majority of veterinary vaccinations that are approved for use in medicine (McVey and Shi, 2010, Unnikrishnan et al., 2012). Because live attenuated vaccines elicit both humoral and cellular immune responses, they have the potential to be very successful. Yet, a significant worry surrounding these kinds of vaccinations is the possibility that the microbe may revert to a virulent phenotype. Attenuated vaccinations may have more efficacy than killed or inactivated vaccines, despite the fact that the former are usually safer. Due of their requirement for complicated components in the culture medium, commercial vaccines based on toxoids (inactivated toxins) have some disadvantages. It is necessary to develop better and safer vaccinations that can prevent, control, or eradicate animal illnesses since the three currently available vaccine types have limits and certain diseases have not yet been effectively treated with an effective vaccine (Dunham, 2002, Redding and Weiner, 2009).

A number of logically created and subunit vaccines have already made it to the veterinary market. Recombinant vaccines provide an appealing method of overcoming the constraints of traditional immunizations. Around the world, work is being done to use recombinant DNA technology to create vaccinations that are more effective against a wide range of illnesses. Recombinant vaccines are created utilizing structure-based design, epitope-focused design, or genomic-based screening, which are based on carefully crafted recombinant highly purified antigens. (Correia et al., 2014, Dellagostin et al., 2011). These molecular approaches have not only improved our understanding of the genes causing virulence and made it easier to identify the factors that determine protective immune responses, but they have also given rise to new techniques for creating innovative vaccines against illnesses that are infectious, parasitic, or metabolic. To guarantee the success of recombinant vaccines, strong and secure adjuvants are required, as the intrinsic immunogenicity of recombinant antigens is frequently lower than that of more conventional vaccinations. Lack of exogenous immune stimulating components is the cause of the poor immunogenicity often seen in recombinant antigens. Adjuvants for recombinant antigens vary, and the immunomodulatory effects of a certain adjuvant when combined with a particular antigen are known.

1.1. Overview of vaccine development and principles

Veterinary vaccines are developed to increase production of livestock including cattle and poultry, improve the health of companion animals, and prevent animal-to-human transmission from domestic and wild animals. Aggressive vaccination strategies globally have eliminated rinderpest, a devastating cattle disease in 2011. This was the first livestock disease, to have ever been eradicated through vaccination efforts, and the global benefits of rinderpest eradication are estimated to be in the billions of dollars (Thomas et al., 2022). Vaccination strategies are required to eliminate

diseases of livestock so as to increase the food security in a rapidly changing world due to climate changes. Increase in temperature will lead to proliferation of pathogens where some species may become more susceptible to a novel pathogen during heat stress (Rojas-Downing et al., 2017). A vaccine is a biological product that can be used to safely induce an immune response that confers protection against infection and/or disease on subsequent exposure to a pathogen. To achieve this, the vaccine must contain antigens that are either derived from the pathogen or produced synthetically to represent components of the pathogen (Pollard & Bijker, 2021).

Early vaccines formulated with whole microbial pathogens supply an extensive repertoire of different epitopes that, in general assure sufficient immune stimulatory activity within an immunogenic heterogeneous population. In contrast, highly purified antigens, probably consisting only of a limited number of epitopes, may pose the risk of insufficient interaction with individuals missing the adequate immune-receptor repertoire (Zepp, 2010). Moreover, genetic heterogeneity of the pathogen may counteract the expected benefit of highly purified vaccine antigens. In other words, while being highly specific towards the pathogen against which it should provide protection, the initiation of the vaccine-related immune response has to be adequate to fulfill this task for every individual of a given population. From its emergence out of immunology, vaccine-logy has focused on understanding the natural, course of an infectious disease especially with regard to the specific steps leading to a protective immune response. In order to learn from nature, as a first step, vaccinology dissects the natural immune response to identify the components most relevant for the protection from infection and/or disease. Because protective immune response mechanisms vary significantly depending on the pathogenesis, of the infectious disease (Table 1) (Zeppo 2010), this task requires profound understanding of the cellular and molecular elements of the human immune system involved.

Over several decades, the approach to vaccine design moved from a time where there was little understanding of how vaccines activate the immune system (even though many of these vaccines are still available today) to the situation now where the understanding of the immune system has underpinned the development of vaccines with higher specificity due also to advances in the manufacture of highly purified antigens. With these advances, a major challenge emerged that needed to be tackled; highly purified and defined antigens can have the unwanted consequence of impaired immunogenicity in comparison with less purified vaccines. From quite early in the history of vaccinology, additional components, adjuvants (from the Latin word *adjuvare*, meaning 'to help or aid'), were introduced in to vaccine formulations to improve the immunogenicity of vaccines. The most commonly used adjuvant today is based on aluminium salts (Brewer, 2006). It is now known that aluminium salts (and other adjuvants) are able to provide pro inflammatory or immune-stimulatory effects as well as prolong the persistence of vaccine antigens by slowing down antigen degradation (Brewer, 2006). However, it is also demonstrated that aluminium salts primarily promote antibody responses, with little or no effect on Th1 and cytotoxic cell immune responses (Zepp, 2010). That is probably the reason why aluminium salts have been used more successfully in vaccines against pathogens where antibodies represent the primary mechanism of protection. For vaccines designed against pathogens where it is important to induce a cytotoxic T cell-mediated immune response, aluminium salts have been found to be inadequate to induce the required protection (Collier, 2017). Recently accumulating knowledge has clarified that this is essentially due to a lack of 'intrinsic immune defense triggers' usually present in the pathogen, such as pathogen-associated molecular patterns (PAMPs) (Zepp, 2010). Naturally available PAMPs may be reduced or even become lost during the selection process for relevant vaccine antigens or in course of the purification processes. PAMPs represent conserved 'danger signals' that are recognized by pattern-recognition receptors (PRRs), mainly of the innate immune system but to some degree also found on Band T cells, including so-called Toll-like receptors (TLRs) (Mogensen, 2009). The triggering of PRRs by PAMPs provides an important early activation signal that is affected by what type of pathogenesis involved, and can alert multiple aspects of the adaptive immune responses, i.e. type, magnitude and quality of specific B and T cell activation, immune memory induction, etc.

Hence, it is by the recognition of particular PAMPs, the innate immune system can act in different immunological environments that can shape the type of protective adaptive immune responses. It was a logical step to target the 'danger-sensing' PRRs in order to improve the quality and persistence of vaccine-related immune responses in all population groups. In the context of vaccine development, this has led to an appraisal of the role of adjuvants as substitutes of natural immune-defence triggers to activate the appropriate DC response (Leroux-Roels, 2010). The deficits of aluminum salts as adjuvants, especially with regard to their capacity to induce cytotoxic T cell-mediated immune responses, have triggered extensive research and effort to develop new adjuvants that can improve immunogenicity as well as the type of adaptive immune response. The most successful examples of these developments are emulsions, virosomes and adjuvant systems, as described in a companion paper. Most recently the improved understanding of the important role of TLR signaling has led to the recognition of a role for immune-stimulatory DNA, such as CpG (Higgins et al., 2007), and other TLR agonists, such as deacylated monophosphoryl lipid A (MPL), as vaccine adjuvants. Moreover, adjuvant systems based on the combination of a classical adjuvant plus immune enhancing molecules, have been developed to modulate aspects of the immune system to help achieve the desired protective response (Garçon et al., 2007).

2. Principles of Vaccine Development

Modern vaccine design builds on the principal concept of inducing protective immunity against a disease by mimicking the naturally occurring immune response, against the disease-causing pathogen, but without inducing the disease. To achieve this, the factors that define the interaction between the human organism and an infectious agent have to be considered at the level of the population, individual, cells and, most recently, genes (Fig. 1) (Hammer et al., 2014). In most instances, vaccines are developed to protect human beings from infectious diseases on a population-based level. This implies that vaccines provide protection for basically every individual within an immunogenetically heterogeneous population.

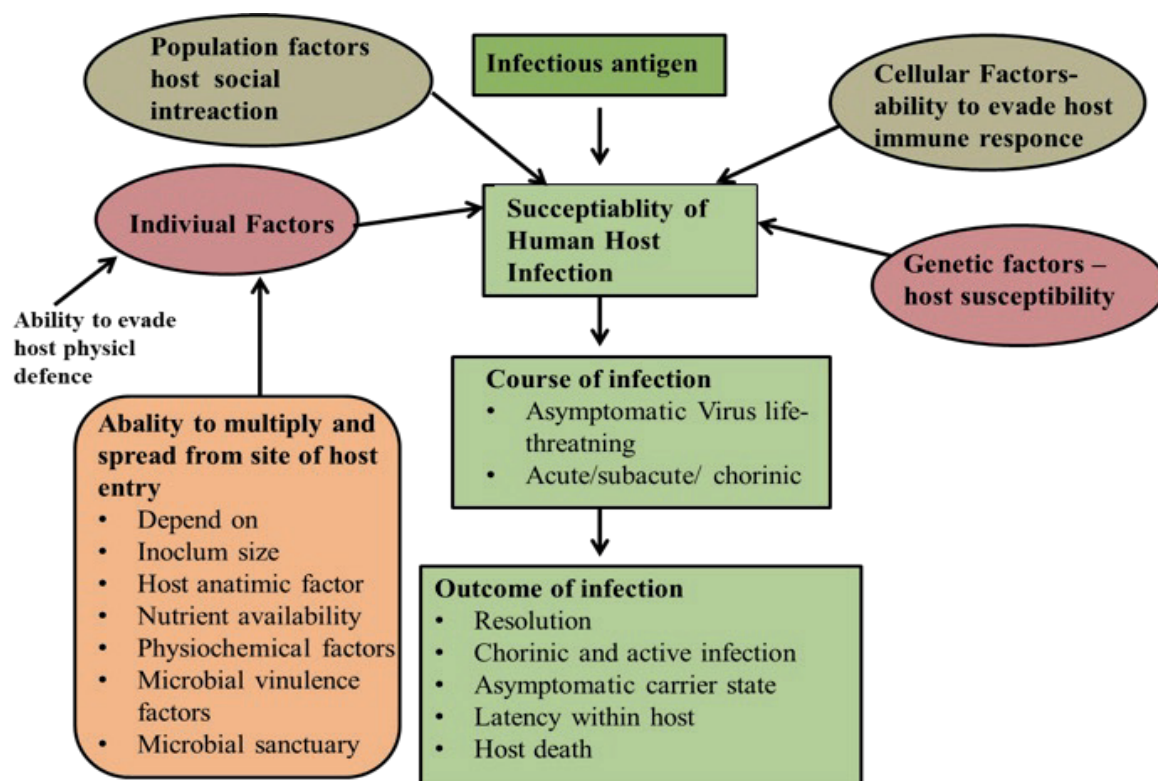


Figure 1 principle of vaccine development

2.1. Immunological basis of vaccination

The immune system is composed of two major populations: B-cells, producing antibodies, and T-cells, mediating cellular immunity. T-cells are divided into CD4 and CD8 subsets and the CD4 T-cells are divided, based on the pattern of cytokine secretion, into Th1, Th2 and Th3 cells. The differences between B- and T-cells are not only functional but are also seen in the mechanism of recognition of antigens. The B-cells, via the Ig receptor, recognize both conformational and linear epitopes directly on the surface of native macromolecules. In certain cases the recognition of epitopes leads to activation and differentiation of B-cells directly, i.e. T-independent antigens. In other cases they need the help of CD4 T-cells, i.e., T-dependent antigens (Broere & van Eden, 2019). The isotype of antibodies is dependent on collaboration with T-cells. Whereas Th1 cells polarize the response to IgG2, the collaboration with Th2 leads to IgG 1 and IgE. The polarization of isotopes is caused by cytokines secreted by these subsets that represent second signals: interleukin-2 (IL-2), and interferon) in the case of Th1 and IL-4, IL-5, and IL-1 in the case of Th2 cells (Ding, 2000). Antibodies exert their protective capacity by blocking the microbial receptor through which they bind to the cellular receptor of permissive cells, promoting phagocytosis via opsonins and complement dependent lysis. In contrast to B-cells, T-cells are unable to recognize the antigens on the surface of native macromolecules. They recognize only fragments of degraded antigens in association with MHC molecules. CD4 T-cells recognize peptides or glycopeptides in association with class II MHC molecules. The peptides are produced from the processing of exogenous proteins in the endosomes of professional antigen-presenting cells (APCs; B-cells, macrophages, and dendritic cells), where they bind to nascent and empty class II molecules (Bona, 2002). The peptide-class II complex is trans located to the membrane, where interaction with the T-cell receptor (TCR) of T cells occurs. Figure 1 illustrates cellular events leading to generation of a class II-peptide complex within professional APCs. CD8 T-cells recognize the peptides in association with class I MHC molecules. The peptides are derived from endogenous proteins, including proteins of intracellular microbes. The proteins are fragmented by proteasomes, and the peptides are bound to transporter proteins (TAPs), and taken to the endoplasmic reticulum (ER), where they are released and bind to nascent class I MHC molecules (Perreault, 2010). The peptide-class I complex is transferred via the Golgi apparatus to membranes and is recognized by CD8 T-cells. The generation of a class I-peptide complex. T-cells or natural killer (NK) cells can recognize lipopeptides or glycopeptides in association with CDI molecules, which are less polymorphic than MHC molecules. The major functions of cells involved in host response to vaccines. Interdisciplinary contributions of accumulated knowledge and new methodologies have led to the development of new vaccines stimulating production of antibodies, cytokines, and cytotoxic responses, contributing to the recovery process from infectious diseases (Bona, 2002).

This also contributed to the development of immunotherapeutic ideally, a vaccine should display the following properties:

1. The antigen should be pure and chemically well defined.
2. It should induce a protective immune response.
3. It should exhibit a constant antigen specificity without being the subject of genetic variation
4. The protection should be lifelong or induced promptly after a booster dose.
5. It should be devoid of side effects.
6. The manufacturing should be inexpensive.

Table 1 Protective immune responses to infection differ according to type of disease.

Sr .No	Disease	Pathogenesis	Immune response
1.	Cholera	Local infection, ente-rotoxin	IgA (mucosa) IgG (neutralization)
2.	Diphtheria	Local infection, exo-toxins	IgG(neutralization)
3.	Hepatitis B	Systemic infection	IgG (elimination of the virus) IFN γ , T cells (infected cells)
4.	Influenza	Local infection Syste-mic infection	IFN γ , IgA,IgG T cells(infected cells)
5.	Tuberculosis	Systemic infection	T cells, IFN γ Macrophages

2.2. Types of vaccines (live attenuated, inactivated, subunit, etc.)

The essential component of most vaccines is one or more protein antigens that induce immune responses that provide protection. However, polysaccharide antigens can also induce protective immune responses and are the basis of vaccines that have been developed to prevent several bacterial infections, such as pneumonia and meningitis caused by *Streptococcus pneumoniae*, since the late 1980s (Pollard & Bijker, 2021). Vaccines are generally classified as live or non-live (sometimes loosely referred to as ‘inactivated’) to distinguish those vaccines that contain attenuated replicating strains of the relevant pathogenic organism from those that contain only components of a pathogen or killed whole organisms (Fig. 2). In addition to the ‘traditional’ live and non-live vaccines, several other platforms have been developed over the past few decades, including viral vectors, nucleic acid-based RNA and DNA vaccines, and virus-like particles. The distinction between live and non-live vaccines is important. The former may have the potential to replicate in an uncontrolled manner in immune-compromised individuals (for example, children with some primary immune-deficiencies, or individuals with HIV infection or those receiving immunosuppressive drugs), leading to some restrictions to their use (Rubin et al., 2014). By contrast, non-live vaccines pose no risk to immune-compromised individuals live vaccines are developed so that, in an immune-competent host, they replicate sufficiently to produce a Finding an immune response that correlates with protection can accelerate the development of and access to new vaccines (Plotkin, 2020) The antigenic component of non-live vaccines can be killed whole organisms (for example, whole-cell pertussis vaccine and inactivated polio vaccine), purified proteins from the organism (for example, acellular pertussis vaccine), recombinant proteins (for example, hepatitis B virus (HBV) vaccine) or polysaccharides (for example, the pneumococcal vaccine against *S. pneumoniae*) (Table.2). Toxoid vaccines (for example, for tetanus and diphtheria) are formaldehyde-inactivated protein toxins that have been purified from the pathogen. Non-live vaccines are often combined with an adjuvant to improve their ability to induce an immune response (immunogenicity). There are only a few adjuvants that are used routinely in licensed vaccines. Vaccines contain other components that function as preservatives, emulsifiers (such as polysorbate 80) or stabilizers (for example, gelatine or sorbitol) (Mitkus et al., 2013). Various products used in the manufacture of vaccines could theoretically also be carried over to the final product and are included as potential trace components of a vaccine, including antibiotics, egg or yeast proteins, latex, formaldehyde and/or gluteraldehyde and acidity regulators (such as potassium or sodium salts) (Eldred et al., 2006).

Table 2 Types of vaccine first introduces (Plotkin 2020).

Sr.No	Type of vaccine	Licensed vaccines Used	First introduced
1.	Live attenuated (weakened or inactivated)	Measles, mumps, rubella, yellow fever, influenza, oral polio, typhoid, Japanese encephalitis, rotavirus, BCG, varicella zoster	1798 (smallpox)
2.	Killed whole organism	Whole-cell pertussis, polio, influenza, Japanese encephalitis, hepatitis A, rabies	1896 (typhoid)
3.	Toxoid	Diphtheria, tetanus	1923 (diphtheria)
4.	Subunit (purified protein, recombinant protein, polysaccharide, peptide)	Pertussis, influenza, hepatitis B, meningococcal, pneumococcal, typhoid, hepatitis A	1970 (anthrax)

3. Adjuvants and delivery systems for enhancing vaccine efficacy

One effective method of avoiding infectious illnesses is vaccination. They function by exposing the body to a weakened or dormant version of a microbe or virus, which aids in the immune system's development of disease resistance. But on their own, some vaccinations fail to elicit a potent enough immune response. Adjuvants and delivery methods are useful in this situation.

Ingredients called adjuvants are added to vaccinations in order to enhance the immunological response.

They function in many ways, including:

- **Drawing immune cells, including antigen-presenting cells (APCs), to the injection site:** This produces a small-scale inflammatory response that draws immune cells to the location. Antigen-presenting cells, or APCs, are white blood cells that absorb and digest the components of viruses or bacteria that the immune system recognizes (Fachada, 2008).

- **Assisting APCs in more efficiently presenting antigens:** After absorbing antigens, APCs fragment them into smaller pieces and display them to other immune cells, such T cells and B cells, on their surface. Adjuvants can aid in the development of APCs and increase their capacity to present antigens (Grødeland et al., 2015).

- **Activating immune cells:** Adjuvants can also directly stimulate the production of more antibodies or other immunological components by immune cells.

Another strategy to increase vaccination effectiveness is through delivery methods. They serve as transporters, shielding the antigen and assisting in its delivery to the appropriate immune system cells. Typical delivery methods include the following:

- **Liposomes** are microscopic spheres composed of lipid molecules that have the ability to encase antigens. Antigens can be directed towards APCs and shielded from destruction by liposomes.

- **Polymer nanoparticles** are artificial polymer-based particles with the ability to encase antigens. It is possible to engineer polymer nanoparticles so that they release antigens gradually over time, perhaps promoting a longer-lasting immune response (Zhou et al., 2023).

- **Viral-like particles**, also known as VLPs, are essentially viral shells that have been modified to contain antigens. Because VLPs may imitate a virus's structure, they may aid in eliciting a potent immune response (Shirbaghaee & Bolhassani, 2016).

- By using adjuvants and delivery systems, scientists can develop more effective vaccines that provide longer-lasting protection against infectious diseases.

Here are some of the benefits of using adjuvants and delivery systems in vaccines:

Enhanced immunogenicity: Vaccines can be rendered more immunogenic, or more likely to elicit a potent immune response, by the addition of adjuvants and delivery mechanisms.

Broader immune reaction: Certain adjuvants have the ability to boost the cellular immune response, which destroys infected cells, as well as the humoral immunological response, which creates antibodies (Schijns et al., 2020).

Lower vaccine dosage: Adjuvants and delivery methods may occasionally permit the use of a lower dose of antigen in a vaccination, hence lowering the possibility of adverse reactions.

Better thermostability: Certain delivery methods can shield antigens from deterioration at high temperatures, which can facilitate the storage and transportation of vaccinations. In general, adjuvants and delivery methods play a significant role in the creation of contemporary vaccines. They may improve the safety, efficacy, and convenience of vaccination administration.

4. Innovations in Vaccine Design

Microbial pathogens have developed complex and efficient ways of counteracting and evading innate and adaptive immune mechanisms. The strategies used by pathogens determine strongly the type of immune response a vaccine should elicit and how the vaccine should be formulated. Improved knowledge of immune response mechanisms has brought successes in the development of vaccines that protect against challenging pathogens as well as vaccines that can be used in immune-compromised and elderly populations. This includes the production of highly purified antigens that provide a better reactogenicity and safety profile than some of the early whole-pathogen vaccines. Successful attempts to improve antigen purity, however, can result in weakened immunogenicity. Modern human vaccine development builds on understanding of the etiology, epidemiology and pathogenesis of the target infection or disease, as well as the target population (Meyer & Zepp, 2022).

4.1. Genetic engineering approaches in vaccine development

The development of vaccines has been transformed by genetic engineering, which provides precise techniques for the creation of efficient and tailored vaccinations. The following is how the creation of vaccines uses genetic engineering:

4.1.1 Subunit Vaccines:

This is a popular method in which genetic engineering techniques are used to introduce a gene expressing a particular antigen (protein that elicits immunity) from a virus or bacteria into a harmless host cell (bacteria, yeast). This approach makes it safer by removing the need to handle the pathogen in its whole. The host cell machinery then creates the antigen, which is purified and utilized in the vaccine. One effective example of a subunit vaccination created by genetic engineering is the Hepatitis B vaccine (Jorge & Dellagostin, 2017).

5.1.2. Viral Vector Vaccines:

In this instance, the gene encoding a desired antigen from another disease is transferred into a weaker virus. By acting as a vector, this altered virus enters the host cells and delivers the antigen. Once within, the antigen is produced by the host cells, which prompts the immune system to identify and mount an attack against the pathogen of interest. Vaccines using viral vectors have the potential to stimulate both cellular and humoral (antibody) immune responses with great efficacy. Two examples of vaccinations using viral vectors are the COVID-19 and the human papillomavirus (HPV) vaccines (Lundstrom, 2020).

4.1.3. DNA Vaccines:

The strategy here makes use of plasmids, which are tiny circular DNA molecules that carry the target antigen's gene. The body is immediately injected with the plasmid DNA. The plasmid is taken up by host cells, which then use its genetic material to create the antigen and start an immunological reaction (Shedlock & Weiner, 2000). While still in the early stages of research, DNA vaccines have the potential to be advantageous due to their simple manufacturing and capacity to stimulate both humoral and cellular immunity.

4.1.4. Reverse Vaccinology:

To find possible targets for vaccines, this method makes use of genetic data from pathogens. To identify genes encoding proteins necessary for the pathogen's survival or infection process, scientists examine the genome of the pathogen. Then, depending on their capacity to elicit immunity these proteins are assessed as possible candidates for vaccination. Reverse vaccinology can be helpful in discovering antigens from novel or complicated diseases and enables a more focused approach to vaccine development (Sette & Rappuoli, 2010). With the use of genetic engineering, vaccinations can be more precisely, safely, and effectively designed. We may anticipate even more developments in this area as research goes on, which will result in the creation of vaccinations against a larger variety of illnesses.

5. Recombinant vaccines and vectored vaccines

When it comes to combating infectious illnesses, recombinant and vectored vaccines are the fruits of genetic engineering's potent tree. Though they employ distinct strategies, they both aim to boost immunity:

5.1. Recombinant Vaccines: The Targeted Approach

Concept is that the antigen, a protein molecule that the immune system recognizes and mounts defenses against, is the precise target that recombinant vaccines aim to deliver.

Method used was by inserting the gene encoding a desired antigen from a virus or bacteria into a harmless host cell, such as yeast or bacteria, scientists can achieve their goals through genetic engineering. This cell produces a lot of the antigen, almost like a factory. After purification, the antigen is added to the vaccine.

Importance:

They pose little danger of infection because they don't contain the entire virus or bacterium.

Precision: They concentrate the immunological response by targeting the particular antigen that confers immunity.

Proven Efficacy: Several effective vaccinations, such as the Shingles (Herpes Zoster) vaccine and the Hepatitis B vaccine has been proved greater efficacy.

5.2. Vectored Vaccines: The Trojan Horse Strategy

The Idea was that Vectored vaccines transfer the genetic information needed to produce the target antigen by using a modified, weakened virus as a carrier. The technique used was genetic engineering, researchers may transfer the gene encoding for an antigen from one disease to a weaker virus (vector). The antigen is subsequently injected into the body's cells by means of this altered virus. The immune system is triggered to identify and combat the target pathogen by the antigen that the cells create after interpreting the instructions.

Importance:

1. **Strong immunological Reaction:** Viral vectors may trigger a strong immunological response that includes both cellular immunity and antibody production. They are also highly effective in delivering the antigen. This is comparable to an illness that occurs naturally, providing robust defence.

2. **Flexible Platform:** Vaccine development can be more effectively accomplished by using the same weakened virus as a vector to transmit antigens from several infections. Examples include the COVID-19 vaccine (AstraZeneca) and the HPV vaccination.

5.3. Key Differences

- **Delivery method:** Recombinant vaccines deliver the purified antigen directly, while vectored vaccines use a modified virus as a carrier.

- **Immune response:** Vectored vaccines often induce a stronger immune response due to the stimulating effect of the viral vector.

- **Development time:** Recombinant vaccines can be faster to develop compared to vectored vaccines, which require engineering a safe and effective vector.

In essence, recombinant vaccines are like giving the immune system a clear picture (the antigen) of the enemy. Vectored vaccines, on the other hand, are like sending an undercover agent (modified virus) carrying the enemy's blueprint (antigen gene) to trigger a more vigorous response. Both approaches have revolutionized vaccine development, offering safe and effective ways to prevent infectious diseases.

6. Nanotechnology and novel delivery platforms

Nanotechnology has emerged as a game-changer in vaccine development, offering innovative ways to deliver vaccines and enhance their efficacy.

What is Nanotechnology?

Nanotechnology deals with manipulating materials at the atomic and molecular level, creating structures in the range of 1-100 nanometers (nm). For comparison, a human hair is about 80,000 nm wide! At this nanoscale, materials exhibit unique properties that can be harnessed for various applications, including medicine.

Nanotechnology offers advantages in delivering therapeutic cargoes for modulating immune cell activity, including selective targeting, controlled release, greater bio distribution, and prolonged effects. It can enhance anti-tumor immunity of immune cells like T cells or NK cells, and "re-educating" tumor-associated immune cells like tumor-associated macrophages (TAMs) is a promising strategy for immunotherapy and immune-engineering. TAMs, classified as M2d, are critical therapeutic targets as they constitute the major immune cell populations in tumors and mediate immunosuppression through IL-10 and TGF- β . Strategies to deplete or re-polarize TAMs have been extensively investigated, and tumor micro environment (TME) reprogramming approach is a promising therapeutic strategy for cancer immunotherapy by enhancing anti-tumor immunity. Combining nanotechnology with immune-modulatory agents, particularly checkpoint blockade and microenvironment reprogramming, is a promising approach for cancer immunotherapy.

6.1. Delivery of protein-based immune-modulators

In recent years, the development of checkpoint inhibitors, particularly monoclonal antibodies (mAbs), has significantly advanced. PD-1, a major immune checkpoint, is expressed by activated T cells, B cells, and monocytes. Cancers often hijack this pathway to evade immune surveillance, enhancing anti-tumor immunity. Despite the clinical success of mAb-based checkpoint blockade therapy, systemic administration can still suffer from premature degradation and off-target toxicities. Several groups have worked on direct delivery of PD-1 antibodies using biodegradable

Nano carriers like PLGA or UCNP. PD-1 antibodies can be co-delivered with other adjuvants or small molecule inhibitors to improve efficacy. Recent advances have focused on triggered-release systems based on tumor microenvironment cues, such as pH, reactive oxygen species (ROS), or matrix metalloproteinase (MMP) expression. A MMP-mediated, biodegradable DNA nano-cocoon has been developed, allowing both CpG-oligodeoxynucleotides (CpG-ODN) and PD-1 antibodies to be co-loaded into the same nanocomplex for preventing postsurgical tumor relapse. The release of the cargoes is mediated by the cleavage of the triglycerol monostearate capsules encasing the restriction enzyme HhaI by MMPs. A separate study utilizing MMP2-mediated degradation co-encapsulated IMD-0354-containing lipid nanoparticles with PD-1 antibodies in a Nano gel to achieve PD-1 blockade and TAM repolarization. This combination approach targeted both T cells and M2 TAMs, leading to significant tumor growth inhibition and extended survival of mice bearing B16 tumors. Other checkpoint inhibitors have been directly delivered or combined with other Nano materials to achieve greater therapeutic efficacy.

6.2. Delivery of nucleic acid-based immune-modulators

Nucleic acids, such as plasmid DNA (pDNA), messenger RNA (mRNA), short interfering RNA (siRNA), and microRNA, have been found to modulate the immune system. The negative charge of nucleic acids allows them to be encapsulated using cationic materials with high efficiency. SiRNA-mediated knockdown has been formulated into various types of nanoparticles composed of lipids, polymers, and inorganic matrices for modulating the target immune cell. RNA therapeutics have a significant advantage over DNA therapeutics for immunotherapy because RNA can function readily in the cytosol, whereas DNA must localize to the nucleus for proper expression. This creates a major barrier for non-viral plasmid DNA delivery into immune cells, including primary T cells, NK cells, DCs, and macrophages.

Recent efforts have focused on developing biodegradable platforms for clinical translation, like protein therapeutics. These biodegradable platforms could consist of either polymeric, lipid, or inorganic materials. A hybrid lipid calcium phosphate nanoparticle (LCP NPs) was developed that encapsulated PD-1 siRNA, resulting in greater killing efficacy and cytokine production. Hanafy et al. developed lipid nanoparticles containing acid-labile PEG linkers for the down regulation of PD-1 on tumor-infiltrating lymphocytes (TAMs). They observed increased uptake of the acid-sensitive PEG lipid nanoparticles in macrophages, leading to reduced tumor size and PD-1 expression. To enhance the efficacy and targeting of TAMs, researchers have focused on key receptors, such as CD163 and CD206. Zhang et al. synthesized a poly (β -amino ester) (PBAE) Nano-carrier coated with poly (glutamic acid)-mannose for the targeted co-delivery of in vitro-transcribed (IVT) IRF5 and IKK mRNA (3:1) in an ovarian cancer mouse model. However, the authors did not observe complete eradication of tumors in the various mouse models tested, suggesting that this approach is best used in combination with other therapies for efficacy.

7. Vaccination Strategies for Livestock

Effective livestock vaccination methods priorities biosecurity measures, follow a well-defined schedule taking age and illness into account, target herd immunity, and customize immunizations to unique farm hazards (core vs. non-core). These strategies help assure healthy herds. By keeping track of vaccines, an all-encompassing strategy for food safety and animal protection may be developed, including tracking and revaccination.

7.1. Herd immunity and vaccination coverage

Although direct protection of individuals through vaccination has been the focus of most vaccine development and is crucial to demonstrate for the licensure of new vaccines, it has become apparent that a key additional component of vaccine-induced protection is herd immunity, or more correctly 'herd protection' (Fine, 1993). Vaccines cannot protect every individual in a population directly, as some individuals are not vaccinated for various reasons and others do not mount an

immune response despite vaccination. Fortunately, however, if enough individuals in a population are vaccinated, and if vaccination prevents not only the development of disease but also infection itself (discussed in more detail below), transmission of the pathogen can be interrupted and the incidence of disease can fall further than would be expected, as a result of the indirect protection of individuals who would otherwise be susceptible. For highly transmissible pathogens, such as those causing measles or pertussis, around 95% of the population must be vaccinated to prevent disease outbreaks (Ruderfer & Krilov, 2015), but for less transmissible organisms a lower percentage of vaccine coverage may be sufficient to have a substantial impact on disease (for example, for polio, rubella, mumps or diphtheria, vaccine coverage can be $\leq 86\%$). variable from season to season and is also confounded by the variability in vaccine effectiveness each year (Wright, 2022). Modest vaccine coverage, of 30–40%, is likely to have an impact on seasonal influenza epidemics, but $\geq 80\%$ coverage is likely to be optimal (Pollard & Bijker, 2021). Interestingly, there might be a downside to very high rates of vaccination, as the absence of pathogen transmission in that case will prevent natural boosting of vaccinated individuals and could lead to waning immunity if booster doses of vaccine are not used. Apart from tetanus vaccine, all other vaccines in the routine immunization schedule induce some degree of herd immunity which substantially enhances population protection beyond that which could be achieved by vaccination of the individual only. Tetanus is a toxin-mediated disease acquired through infection of breaks in the skin contaminated with the toxin-producing bacteria *Clostridium tetani* from the environment so, vaccination of the community with the tetanus toxoid will not prevent an unvaccinated individual acquiring the infection if they are exposed. As an example of the success of herd immunity, vaccination of children and young adults (up to 19 years of age) with capsular group C meningococcal vaccine in a mass campaign in 1999 resulted in almost complete elimination of disease from the UK in adults as well as children (Borrow et al., 2017). Currently, the strategy for control of capsular groups A, C, W and Y meningococci in the UK is vaccination of adolescents, as they are mainly responsible for transmission and vaccine-mediated protection of this age group leads to community protection through herd immunity (Pollard & Bijker, 2021). The HPV vaccine was originally introduced to control HPV-induced cervical cancer, with vaccination programs directed exclusively at girls, but it was subsequently found to also provide protection against HPV infection in heterosexual boys through herd immunity, which led to a marked reduction in the total HPV burden in the population (Brisson et al., 2016).

7.2. Vaccination schedules and timing

For most vaccines that are used in the first year of life, 3–4 doses are administered by 12 months of age. Conventionally, in human vaccinology, ‘priming’ doses are all those administered at less than 6 months of age and the ‘booster’ dose is given at 9–12 months of age. So, for example, the standard WHO schedule for diphtheria–tetanus–pertussis-containing vaccines (which was introduced in 1974 as part of the Expanded Program on Immunization) consists of 3 priming doses at 6, 10 and 14 weeks of age with no booster (Liang, 2018). This schedule was selected to provide early protection before levels of maternal antibody had waned (maternal antibody has a half-life of around 30–40 days, so very little protection is afforded to infants from the mother beyond 8–12 weeks of age) and because it was known that vaccine compliance is better when doses are given close together (Healy et al., 2020). However, infant immunization schedules around the world are highly variable few high-income or middle-income countries use the Expanded Program on Immunization schedule and were largely introduced with little consideration of how best to optimize immune responses. Indeed, schedules that start later at 8–12 weeks of age (when there is less interference from maternal antibody) and have longer gaps between doses (8 weeks rather than 4 weeks) are more immunogenic. A large number of new vaccines have been introduced since 1974 as these have generally been fitted into existing schedules without taking into account the optimal scheduling for these new products. It should also be noted that surveys show vaccines are rarely delivered on schedule in many countries and, thus, the published schedule may not be how vaccines are actually delivered on the ground. This is particularly the case in remote areas (for

example, where health professionals only visit occasionally) and regions with limited or chaotic health systems, leaving children vulnerable to infection (Healy et al., 2020).

8. Challenges and Solutions in Vaccine Development for Livestock

Livestock vaccines face unique challenges due to their diverse immune responses, cost-effectiveness for farmers, and specific diseases. The complex immune systems of livestock require multiple vaccinations or adjuvants, and developing vaccines for diseases in developing countries may not be commercially attractive for pharmaceutical companies. Additionally, thermo stability issues can hinder vaccines in regions with limited cold chain infrastructure. To overcome these challenges, research is focusing on novel platforms like recombinant technology and nanoparticles to create more immunogenic and thermo stable vaccines. Collaborations between public and private sectors are crucial to prioritize vaccines for neglected diseases and ensure affordability in resource-limited settings. Innovation in delivery methods, such as single-dose or mucosal vaccines, can further improve livestock vaccination programs.

8.1. Antigenic variation and pathogen diversity

Infectious diseases are primarily caused by Antiviral Viruses (AVPs), which escape immunity through changes in molecular structures recognized by antibodies or T cells. The development of vaccines against AVPs presents an unprecedented challenge, as researchers must consider the mechanism of action of immune cells and the complex interaction between these cells and rapidly changing epitopes in APVs and cancer cells. CD8C T cells are key components of the immune response against many intracellular pathogens and tumors, and effective vaccines against AVPs will likely need to induce broad and potent cellular immune responses (Servín-Blanco et al., 2016).

The observation that whole protein antigens (Ags) are not necessarily essential for inducing protective immunity has led to the emergence of "structural vaccinology." Structure-based vaccines are designed on the rationale that suitable epitopes should be sufficient to induce protective immune responses against pathogens, including AVPs. Epitope recognition in MHC-restricted T-cell responses involves two different binding events: small peptides bind to MHC molecules after Ag processing, and the resulting peptide-MHC (pMHC) complex is bound by T-cell receptor (TCR) leading to cell activation (Wu et al., 2019).

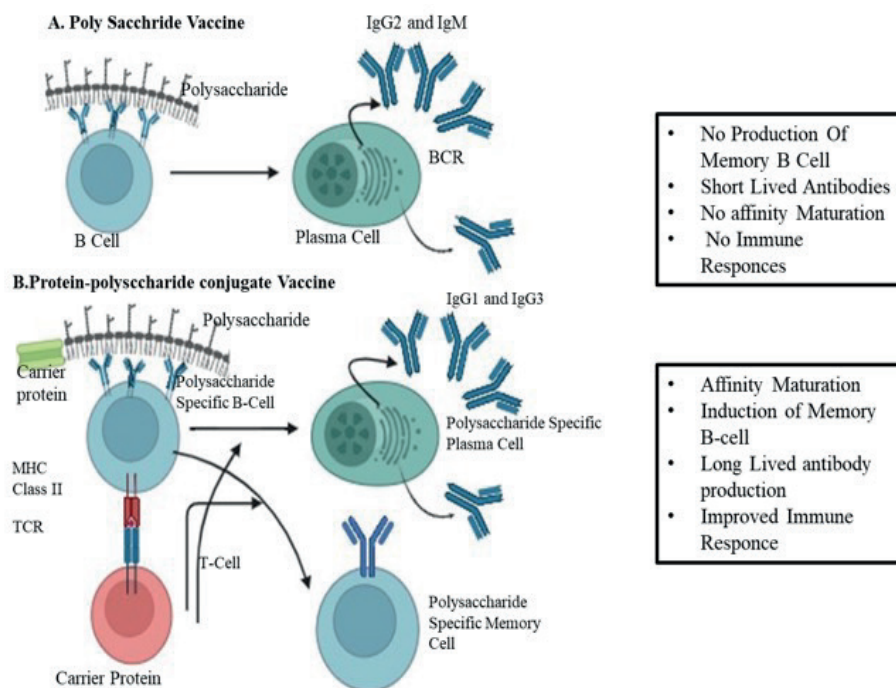


Figure 3: Immune Response of Vaccine

Unprecedented TCR promiscuity explains how a relatively small number of T cells provide effective immune recognition of all possible peptides or why TCRs are cross-reactive. Combinatorial synthetic peptide libraries were used to elucidate the nature of TCR promiscuity, explaining why TCR/MHC interactions are of low affinity and degenerate (Trenth, 2018).

Another important emerging challenge faced by scientists is the presence of a large number of potential epitopes in viral, tumor, and model systems, encoded by short non-primary open reading frames (sORF) and referred to as defective ribosomal products (DRiPs). These "unexpected" epitopes may be targets of a significant portion of yet unappreciated cellular immune responses. AVPs (Antiviral Virulence Factors) have been identified as key factors in the development of universal safe anti-influenza vaccines. Current influenza vaccines rely on inducing antibodies specific for hemagglutinin (HA) and neuraminidase (NA), but due to antigenic variability and immune evasion, yearly vaccinations are necessary. A universal influenza vaccine has been an important objective for decades, but many failed attempts have been made. Recent studies have shown that memory cytotoxic T lymphocytes (CTLs) specific for conserved epitopes of the novel H7N9 influenza A virus can elicit strong CTL responses against any human influenza A virus. However, specific HLA alleles expressed by certain ethnic groups, such as indigenous Australian and Alaskan populations, were associated with poor CTL responses, indicating additional challenges for vaccine development (Hensen et al., 2021).

Recent results highlight the need to design novel high-impact CTL inducing vaccines with greater capacity to suppress antigenic drift, as prior population immunity may reduce the expected impact of vaccines for pandemic influenza control. Successful preclinical studies have shown that vaccination of mice with a mixture of virus-like particles (VLPs) individually displaying four different HAs offered significant protection against a variety of influenza A viruses. Stable HA stem Ags, bearing smaller versions of full-length HA called "mini-HAs" or HA stem mimics, were engineered and shown to protect mice in lethal heterologous and hetero-subtypes challenge models and reduce fever after sub-lethal challenge in cynomolgus monkeys.

DENV, the etiologic agent of dengue fever, has also faced challenges due to high genetic/antigenic variability and the existence of four viral serotypes. The outcome of DENV infection depends on the balance between favorable and unfavorable immune responses, and specific Abs and T cells induced by vaccination may have both protective and detrimental effects. A live-attenuated tetravalent vaccine (DENGvAXIA) containing antigens from all four DENV serotypes has been licensed recently, addressing OAS to some extent. Further research is warranted to develop a universal vaccine against HCV, which causes most acute and chronic liver diseases worldwide (Silva, 2023).

9. Vaccine efficacy in diverse livestock populations

Vaccination has long been considered the most effective defense against infectious diseases, with the primary goal of improving animal health and preventing or reducing pathogen transmission to mitigate the impact of infectious diseases on livestock production. However, the effectiveness of available vaccines is contentious, as they often only confer limited sterilizing immunity, may not prevent infection, and may only partially reduce pathogen transmission. An effective vaccine should reduce within-host pathogen burden, prevent or alleviate disease-induced clinical signs, and improve the general health conditions of exposed animals (Yadav et al., 2020).

Different desirable properties of an effective vaccine include high safety, high sterilizing immunity against a wide range of variant pathogen strains, fast onset of protection, high immunogenicity, and vaccine responsiveness in a broad range of hosts. However, few vaccines on the market satisfy all of these properties. These properties play a crucial role in pathogen transmission and vaccine effectiveness on a population level. Sterilizing immunity affects a host's susceptibility to infection with a heterologous strain, while the impact of a vaccine on pathogen shedding affects an individual's infectivity. Vaccines that accelerate host recovery reduce pathogen transmission by reducing the

infectious period of a host. However, delay in onset of protection or host heterogeneity in vaccine response limit the time or extent of effective vaccine coverage in a population, enabling continued pathogen transmission (Yadav et al., 2020). The effectiveness of a given vaccine in the field depends on the properties of the vaccine itself, how the vaccine is applied, and other biosecurity measures in place. For example, herd closure during a disease outbreak is promoted as a highly effective disease control strategy, while continuous influx of new susceptible, possibly non-vaccinated individuals contributes to long-term disease persistence in a herd (Opriessnig & Doeschl-Wilson, 2019).

Common vaccination strategies for livestock diseases include prophylactic and reactive mass vaccination. Prophylactic vaccination is applied before introducing a pathogen into a herd to prevent recurrence of disease outbreaks or to minimize the risk of a major epidemic. Reactive vaccination, although less effective than prophylactic vaccination, is typically used to control ongoing epidemics. However, the application of either strategy is often hindered by insufficient vaccine availability, economic reasons, safety restrictions, and logistic delays. These factors affect the frequency and timing of vaccination and the effective vaccine coverage in a population (Opriessnig & Doeschl-Wilson, 2019). There is currently no theoretical framework to systematically assess how different vaccine properties and vaccination strategies influence infection invasion and transmission in herds with different demographics. This makes predictions of vaccine effectiveness in the field extremely difficult. For example, the Porcine Reproductive and Respiratory Syndrome (PRRS), caused by the PRRS virus (PRRSv), is an infectious disease that requires a comprehensive framework that combines diverse factors compromising vaccine effectiveness (Johnson, 2009). PRRS is one of the most significant and costly swine diseases globally, with estimated costs per year over \$650 million in the United States and almost 1.5 billion € in Europe. Failed vaccination programs have raised an urgent demand for more effective vaccines, and concerns have been raised about the epidemiological consequences of vaccination and the evaluation of vaccination effectiveness.

PRRS vaccines, categorized into modified-live virus (MLV) and inactivated or killed virus (KV), have not been fully effective in preventing the spread of the virus within a herd. MLV vaccines, which are attenuated live vaccines, have shown delayed but effective protection against homologous and some heterologous PRRSv strains. However, their limited sterilizing immunity and immunogenicity to many circulating PRRSv strains have raised concerns about vaccine effectiveness (Zhou et al., 2021). KV vaccines, containing adjuvants, are known for their high safety but have limited sterilizing immunity against homologous or heterologous PRRSv strains. They often fail to significantly reduce clinical signs, viremia, and duration of shedding in naïve animals. In PRRSv-negative pigs, KV vaccines have failed to elicit detectable antibodies and barely elicit cell-mediated immune response. In PRRSv-positive pigs, reactive vaccination has been reported to strengthen both types of immune responses to the infecting virus, speeding up recovery and potentially reducing infectivity (Bitsouni et al., 2019). Efforts over the last three decades to understand PRRS pathogenesis and vaccinology have been tremendous, but effective PRRS vaccines with safety, broad sterilizing immunity, and high immunogenicity are still lacking. Little is known about how existing vaccines affect virus transmission in a herd or how they could be most effectively applied to prevent or reduce PRRS outbreaks. Mathematical models have proven powerful tools for assessing the combined effects of several interacting factors on virus transmission and predicting the outcome of different types of vaccines or vaccination strategies (Lunney et al., 2016).

10. Future Directions in Livestock Vaccine Development

Looking further into the future, we can expect many more advances in vaccinology and new licensed vaccines, not only against infectious diseases but also against other diseases or chronic disorders not necessarily associated with an infectious pathogen. With therapeutic cancer vaccines, there have been promising results with a number of spontaneous tumor animal models, including models of breast, prostate, pancreatic and colon cancer (Ostrand-Rosenberg, 2004), involving antigen-specific vaccines and DC vaccines formulated with patients' Ds loaded with

tumor- associated antigens (Tagliamonte et al., 2014). Also, several cytokines are being valued as cancer vaccine adjuvants, most notably GM-CSF. DC vaccines and ASCI (antigen-specific cancer immuno therapeutics) represent the most advanced approaches in cancer immuno therapy. DC vaccines work by isolating and culturing the cancer patient's DCs in the laboratory. When in culture, the DCs are exposed to compounds that include tumors associated antigens, so that upon their introduction to the patient, these DCs promote a cytotoxic T cell response against the tumors. The DC vaccine targeted against prostate cancer, Provenge® (sipuleucel-T; Dendreon Corp, Seattle, USA), has recently been licensed by the FDA (Cheever & Higano, 2011). ASCI includes a potential novel class of compounds that target antigens that are selectively expressed by tumor cells, this approach is aimed at teaching the patient's immune system to fight cancer. One of the lead tumor antigens used in the ASCI approach is MAGE-A3 that is expressed in a variety of tumors such as melanoma, NSCLC, bladder and hepato carcinoma. MAGE-3 has been formulated with an Adjuvant System selected for its stability to induce high antibody and robust T cell responses. This formulation has shown encouraging results: proof of concept has been established in phase II studies on Non-Small- Cell-Lung-Cancer (NSCLC) and melanoma, and the Phase III studies are under way on both cancers (Brichard & Lejeune, 2008). A different approach is tried with an epidermal growth factor vaccine against NSCLC. It targets the epidermal growth factor receptor which is over expressed in up to 80% of NSCLC patients and associated with resistance to cytotoxic agents (Vinageras et al., 2008). Allergic diseases affect up to 25% of the population in western countries and may result in life-threatening allergic reactions. Novel immunotherapies are currently under development. Among them a vaccine including aTLR-9 agonist (Broide, 2009).

A phase 2 study with a ragweed allergen conjugated to immune stimulatory DNA (CpG) showed reduction in symptoms of allergic rhinitis during the ragweed season (Creticos et al., 2006), but further studies are needed to confirm these observations. Advances are also expected to treat autoimmune diseases like, type 1 diabetes, arthritis, Alzheimer's disease, or multiple sclerosis. Encouraging results with DNA vaccines have been found in Phase 1 and 2 studies for multiple sclerosis and type 1 diabetes, with intravenous immune globulin. A Phase 1 study for Alzheimer's disease (Relkin et al., 2009). The continuing progress in vaccine technologies and in the understanding of the mechanisms underlying the immune response is producing a more and more refined approach to vaccine design tailored to the desired effect of combating disease.

11. Conclusion

Vaccine development for livestock aims to prevent animal illnesses and improve companion animal health. Traditional immunizations are based on cell membrane chemicals, toxoids, or live microorganisms. Recombinant vaccines use structure-based design, epitope-focused design, or genomic-based screening to overcome limitations. Vaccines are developed to increase livestock production, improve companion animal health, and prevent animal-to-human transmission. Modern vaccine design mimics the naturally occurring immune response without inducing the disease. Adjuvants like aluminum salts have been introduced to improve immunogenicity. Vaccines are classified as live or non-live, and contain adjuvants and delivery systems to enhance their immunological response. These factors improve the safety, efficacy, and convenience of vaccination administration, leading to more effective vaccines. Vaccine design has evolved due to microbial pathogens' complex immune mechanisms, leading to the development of vaccines for challenging pathogens, immune-compromised, and elderly populations. Genetic engineering approaches, such as subunit, viral vector, DNA, and reverse vaccinology, have revolutionized vaccine development. Nanotechnology has also played a significant role in vaccine development, offering innovative ways to deliver vaccines and enhance efficacy. Livestock vaccination strategies prioritize biosecurity measures, follow a well-defined schedule, target herd immunity, and customize immunizations to unique farm hazards. Livestock vaccines face unique challenges due to their diverse immune responses, cost-effectiveness, and specific diseases. Research focuses on novel platforms and collaborations between public and private sectors to improve livestock vaccination programs.

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