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An overview of recombinant vaccines against viral, fungal, bacterial and parasitic diseases

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ABSTRACT

In the present era, particularly with the outbreak of infectious diseases including viral, fungal, parasitic, and bacterial infections, the need for designing and producing vaccines has become more critical than ever. Vaccination is one of the greatest public health achievements of the past century, protecting and improving the quality of life for people worldwide. Recombinant vaccines, produced using recombinant DNA technology, are discussed in this study. The microorganisms causing infections in humans, birds, and animals include adenovirus, infectious laryngotracheitis virus, human papillomavirus, Brucella and Listeria, Candida, Aspergillus, Cryptococcus, and Listeria. Vaccination is a highly effective strategy for controlling these types of diseases, making the acceleration of vaccine development essential for combating these diseases. Since one of the main factors contributing to the increased frequency and severity of diseases is the lack of cellular or humoral immunity, vaccine strategies must be safe and effective in hosts with both healthy and compromised immune systems. Advances in proteomics and systems biology have facilitated the development of several proposed vaccines, particularly as these vaccines enable the localization of proteins and the description of their changes, functions, and interactions.

Introduction

Today, extensive research is being conducted on recombinant vaccines. A recombinant vaccine is a type of vaccine produced through recombinant DNA technology. The production and design process of recombinant vaccines involves inserting DNA encoding the antigen (such as a bacterial surface protein) that induces an immune response in mammalian cells. Ultimately, by creating an immune response, the antigen of the targeted microorganism is produced in the individual's body cells. Currently, several of these recombinant vaccines are FDA-approved, and many others are undergoing clinical trials. In this review article, we will discuss recombinant vaccines based on viruses, fungi, bacteria, and parasites.

1. Methodology:

To find relevant materials for this study, keywords and phrases such as Infectious diseases, *Listeria*, *Candida*, *Adenovirus*, *Leishmania*, Recombinant vaccine, Disease control.were searched in electronic databases including NCBI, Science Direct, PubMed, Scholar Google, and SID. After reviewing the existing studies, approximately 40 articles from the period of 2012 to 2024 were selected for study. After removing duplicates and screening, a total of 30 of the most relevant articles were reviewed. Initially, 40 articles were retrieved in the first stage of database search. After removing duplicates, 35 articles remained for abstract and title review. Subsequently, 5 articles were excluded due to insufficient relevance and information. Ultimately, 30 of the most relevant articles were accepted, and after thorough screening and review of the full text, they were utilized for this study.

2. Findings:

Recombinant Vaccines: Vaccination is a highly effective strategy for disease control. Vaccinating 18-day-old embryos through in ovo inoculation during incubation is a method to combat various diseases. To this end, conventional and specific strains were examined in vivo. Molecular engineering technology can produce new vaccines called recombinant vaccines that can be used in vivo or by subcutaneous injection in one-day-old chicks. Recombinant vaccines present a new opportunity to develop control measures and eradicate diseases in poultry facilities. These vaccines consist of a vector (including viruses or bacteria) that expresses foreign antigens. These processes involve inserting genes encoding antigens, such as proteins, into the vector genome, and when it replicates, it expresses a product with the inserted fragments.Vaccination with recombinant vaccines results in an immune response against the vector, but also against the antigens recognized by the vector without the actual disease agent, thus eliciting an immune response. Recently, several recombinant vaccines have been introduced to the commercial poultry market, offering new strategies for each specific area. These vaccines provide a similar level of prevention as conventional vaccines and enhance both cellular and humoral immunity (Sanders & Moore, 2021).

Vaccination Objectives: Vaccines can be classified into several different types, but ultimately they all work on one principle: stimulating an immune response to recognize a pathogen (a disease-causing organism) or part of a pathogen. Once the immune system is trained to recognize this, if the body later encounters the pathogen, it will be eliminated. Specifically, the immune system recognizes "antigens," parts of the pathogen on its surface or inside it, which are not usually found in the body under normal circumstances (Hein et al, 2021). Simply put, a vaccine is a biological preparation that provides active acquired immunity to a specific disease. Typically, a vaccine consists of a biological agent representing the disease-causing microorganism. It is often made from

a weakened or killed form of the microorganism, its toxins, or one of its surface protein antigens(Abdelaziz et al, 2024).

Types of Vaccines: While various vaccines can be created using recombinant DNA technology, recombinant vaccines can be broadly classified into two major categories:

1. DNA Vaccines (DNA-based vaccines)

2. Recombinant Protein Subunit Vaccines (Protein-based vaccines) (Cox, 2021).

1. DNA Vaccines: These vaccines usually consist of synthetic DNA containing a gene that encodes the disease-causing protein. Typically, plasmid DNA used as a vaccine is replicated in bacteria such as E. coli, isolated, and purified for injection. This naked DNA is usually injected intramuscularly or intradermally. The principle behind DNA vaccines is that the antigen can be expressed directly by the host cells, simulating a viral infection and eliciting an immune response from the host. This is similar to GenScript's DNA Immunization Technology, a powerful tool that helps generate specific antibodies against membrane proteins and other challenging antigens. It is also used in early studies of DNA vaccine development. The DNA immunization technique allows antigen production to occur within the body, eliminating the need for protein antigen production and purification in vitro.DNA vaccines have been used to express antigens from many pathogens, such as influenza, HIV, malaria, and tuberculosis, leading to immune responses against these etiological agents in several animal models(Lim et al, 2020). However, DNA vaccines have shown lower immunogenicity in non-human primates and humans, even though they have proven to be safe and well-tolerated. DNA vaccines are similar to inactivated virus vaccines and recombinant vaccines in some respects. However, because DNA vaccines do not cause infections in organisms, they are distinct from live virus vaccines. Additionally, unlike DNA vaccines, live virus vaccines may suppress the immune system, be less effective in providing immunity due to attenuation, cause complications in pregnant women or immunocompromised individuals, or be harmful due to contamination during production.Nucleic acid vaccines are recognized as the fifth type of vaccines or third-generation vaccines (following inactivated vaccines, purified pathogen proteins, recombinant vaccines, and viral vector vaccines).(Gray & Weiner, 2020).

2. Recombinant Protein Subunit Vaccines: These subunit vaccines contain only part of the pathogen. Often, they are synthetic peptides representing a protein component that elicits an immune response. They can also include protein subunits (antigens) expressed in a heterologous expression system (E. coli, yeast, insect, etc.) using recombinant protein expression technologies. Most vaccines under investigation today are based on such purified recombinant proteins or antigenic subunits. Prokaryotic expression systems for vaccine antigen production include bacteria like E. coli, and eukaryotic systems include mammalian cells, yeasts, or insects.Several factors are considered before selecting the appropriate system for vaccine antigen expression. Expression levels, selection markers, and the presence or absence of post-translational modification processes are essential factors influencing the efficiency of recombinant antigen production as a vaccine. Bacterial expression systems are widely used due to their ease of use and high expression capacity.Choosing the right antigen is one of the most critical steps in any antibody-related project. In most cases, scientists use either protein antigens or peptide antigens for this purpose. Given their structural complexity and size, proteins are inherently strong immunogens Additionally, antibodies generated from protein antigens recognize multiple structural epito(Lim et al, 2020). pes on the target protein. Recombinant protein expression in E.coli is often used to produce protein antigens for antibody production.Antibody fragments are engineered antibodies containing only part of an immunoglobulin. One example of an antibody fragment group is the antigen-binding fragment or Fab, the antibody region that binds to antigens. This type of antibody fragment consists of a constant domain and a variable domain from each heavy and light chain. The second type of antibody

fragment group is the Fc region, considered the antibody's tail region that interacts with cell surface receptors and complement system proteins.Although these two main groups of antibody fragments exist, there are several other types. For example, scFvs and VHH/VH are also used for various research applications. scFvs and VHH/VH retain full antigen-binding capacity and are used for research, diagnostic, and therapeutic purposes. Antibody fragments are particularly useful in applications where epitope binding alone is sufficient for the desired effect, such as therapeutic applications like vaccine development through virus neutralization or receptor blocking. Given that E. coli has a simple system and is easily scalable using fermentation, it becomes the best system for expressing antibody fragments, especially for vaccine research. Recombinant vaccines, such as the Hepatitis B vaccine and the HPV vaccine, are among these (Gray & Weiner, 2020).

There are four main types of vaccines:

1. Live-attenuated vaccines
2. Inactivated vaccines
3. Subunit, recombinant, polysaccharide, and conjugate vaccines
4. Toxoid vaccines (Hederman & Ackerman,2023).

Description of Vaccine Types:

1. Live-attenuated Vaccines: These vaccines contain live, but weakened, viruses or bacteria. These microorganisms replicate multiple times in the body and induce an immune response without causing disease. Examples include vaccines for chickenpox, measles, and mumps (Sanders & Moore, 2021).

2. Inactivated Vaccines: These vaccines contain viruses or bacteria that are not alive. Examples include vaccines for polio and influenza (Sanders & Moore, 2021).

3. Subunit Vaccines: These vaccines stimulate the immune system by presenting proteins or sugars derived from the disease-causing microorganism. Subunit vaccines can be further categorized into three types (Sanders & Moore, 2021).

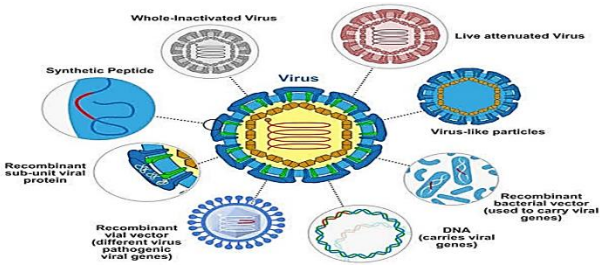
3-1-Protein-based Vaccines: These vaccines are based on proteins, including parts of the microorganism such as inactivated bacterial toxins. Examples include vaccines for diphtheria and tetanus (Sanders & Moore, 2021).

3-2-Polysaccharide Vaccines: These vaccines consist only of polysaccharide molecules found on the outer part of certain bacteria. Examples include vaccines for pneumococcal and typhoid infections (Sanders & Moore, 2021).

3-3-Nucleic Acid-based Vaccines (DNA & RNA): These vaccines use the host's cells to produce the antigen. Examples include vaccines for influenza and coronavirus (Sanders & Moore, 2021).

Distinctive Feature of Recombinant Vaccines Compared to Other Types: Recombinant vaccines are generally safer than other types because they do not contain the microorganism or its genetic material (Sanders & Moore, 2021). As shown in Figure 1, recombinant vaccines can be created using various methods.

Figure 1: Types of Viral Vaccines



Recombinant Viral Vaccines:A viral vector vaccine is a type of vaccine that uses a viral vector to introduce genetic components that produce the antigens of a pathogen, such as a virus, into the host's body. Upon entering the host's body, the designed genetic materials in these vaccines begin producing the pathogen's antigens, which subsequently triggers the production of the desired antibodies in the host. This process prepares the body to resist the pathogen in any future encounter. Viral vector vaccines use a modified version of a harmless virus to humans as a vector to deliver the nucleic acid that codes for the pathogen's antigens into the body. In these vaccines, the virus used as the vector does not cause disease, and the genetic materials sent do not integrate with the host's genome. Viral vector vaccines activate the expression of the virus antigen in the body and, unlike subunit vaccines that only induce a humoral immune response, they also elicit a cytotoxic T lymphocyte response. Viral vectors are generally designed to be replication-deficient, with the genes needed for virus replication removed during vaccine design (Maccann et al, 2022).

The basic principle of viral vaccines is to take a small piece of DNA from the virus, bacteria, or generally the antigen that we want to protect against and introduce it into the producing cells. For example, to produce the hepatitis B vaccine, a part of the DNA of the hepatitis B virus is inserted into the DNA of yeast cells. These yeast cells, capable of producing one of the surface proteins of the hepatitis B virus, are then purified and used as the active ingredient in the vaccine (Cox, 2021).

Different Vectors: The main viral vectors used for developing recombinant vaccines include the turkey herpesvirus (HVT) and poxvirus species. These viruses have large genomes sufficient to accommodate large insertions. Examples of recombinant vaccines include:

- ✓ Turkey herpesvirus expressing the Newcastle disease virus protein
- ✓ Turkey herpesvirus expressing the infectious laryngotracheitis virus protein in poultry
- ✓ Turkey herpesvirus expressing the infectious bursal disease virus protein (Gumboro disease)
- ✓ Fowlpox virus expressing the influenza virus protein
- ✓ Fowlpox virus expressing the Newcastle disease virus protein
- ✓ Fowlpox virus expressing the infectious laryngotracheitis virus protein (Maccann et al, 2022).

Advantages and Characteristics: The main advantages of using recombinant vaccines are that they eliminate specific reactions that occur after vaccination based on live organisms and also eliminate general reactions caused by inactivated vaccines. Using recombinant vaccines based on sequential virus replication (such as herpesvirus) can remove the need for booster vaccinations with live or inactivated vaccines on farms. Additionally, recombinant vaccines allow the use of the DIVA system (Differentiating Infected from Vaccinated Animals). The virus used in the recombinant vaccine cannot combine, and recombinant vaccines eliminate interference with maternal immunity or other vaccines, and delay the virus's action in vaccinated birds (Sanders & Moore, 2021).

Establishing Proper Immunity: The development of immunity using a recombinant vaccine depends on the performance of the vector. It depends on the time required for the vector to create an appropriate level of immunity in vaccinated birds(Landstrom, 2021). The absence of adequate immunity in the early life phase of birds can be compensated for by using a complete recombinant vaccine. When using serological methods (such as HI or ELISA), it should be noted that using a recombinant vaccine does not increase antibody levels against infectious agents. If a high antibody level is achieved, its source could be other complex vaccine viruses or farm sources (Maccann et al, 2022).

Recombinant Viral Vaccines and Genetic Changes: Claims widely shared on social media suggest that recombinant vaccines, such as those developed against *COVID-19*, cause genetic changes in humans. These claims are scientifically incorrect. According to the World Health Organization (WHO), a DNA vaccine involves directly introducing a plasmid containing the DNA sequence encoding the antigen(s) into the appropriate tissues, relying on in situ production of the target antigen. This means that, unlike more conventional vaccines that use a whole pathogen or its fragment, a DNA vaccine involves injecting a small portion of the virus's genetic code (DNA or RNA) to stimulate an immune response without infection. This approach does not create a genetically modified organism, which the Food and Agriculture Organization (FAO) defines as an organism whose genetic material has been altered with one or more genes (called transgenes) using recombinant DNA technology from another organism.In a laboratory study under modeled conditions using liver cancer cells, the entry of Pfizer vaccine mRNA into human cells was investigated. They tested three doses of the vaccine with specified doses on cancer cells over a week. This study reported, "We present evidence that the mRNA *COVID-19* vaccine (Pfizer) can enter human liver cell lines in vitro. mRNA is reverse transcribed into DNA within 6 hours post-exposure to the vaccine. One possible mechanism for reverse transcription is through endogenous reverse transcriptase LINE-1, and this vaccine increases the distribution of LINE-1 nuclear protein." However, the question remains, are these findings reliable? This study is an in vitro experiment, which differs significantly from conditions within the human body, necessitating further tests for accurate and generalizable conclusions. Naturally, only DNA is transcribed to RNA and not vice versa, except by reverse transcriptase enzymes found in retroviruses, which were used in this study but are absent in the human liver. One reason for storing the vaccine at low temperatures is that the RNA's lifespan within the vaccine is short and rapidly degrades into small fragments in the body, meaning that such vaccines exert their effect at the injection site and likely do not reach the liver. Additionally, this study was conducted on cancer cells, not normal cells. As cancer cells exhibit high growth and proliferation rates, they have elevated gene expression, making the results less representative of typical conditions (Ebenig et al, 2022).

Mark Lynas at Cornell University has dismissed the idea that DNA vaccines can cause genetic changes in organisms. Lynas told Reuters that no vaccine can genetically alter human DNA. He said, "This is just a myth, often deliberately spread by anti-vaccination activists to create confusion and distrust." Genetic modification involves deliberately inserting foreign DNA into the nucleus of human cells, and vaccines simply do not do this. Vaccines work by training the immune system to recognize a pathogen when it tries to infect the body, mostly by injecting viral antigens or weakened live viruses to stimulate an immune response through the production of antibodies(Ebenig et al, 2022). mRNA vaccines have previously been investigated for influenza, Zika, rabies, and cytomegalovirus, and the facts about them are:mRNA vaccines do not contain live viruses and do not pose a risk of causing disease in vaccinated individuals.mRNA never enters the nucleus of the cell where genetic material is located, and thus, these vaccines do not affect or interact with our genetic material and genes (Pardi et al, 2020).

Role of Plasmid: By removing parts of the plasmid, it became possible to insert larger genes into the plasmid. Smaller plasmids have several advantages. As a general rule, transformation efficiency is

inversely dependent on plasmid size. When plasmid size exceeds 15,000 base pairs, it acts as a limiting factor; hence, smaller plasmids that can accommodate larger genes are more desirable. Additionally, larger plasmids can create problems with determining the enzyme map and working with restriction enzymes and are replicated in fewer copies, thus producing less DNA. The vaccine can be administered by three methods: inhalation, intramuscular injection, and intradermal injection. Some studies have shown that the injection site can influence the type of induced response. For instance, an experiment showed that when a type of DNA vaccine was administered intramuscularly, it induced a Th1 response, but when the same vaccine was injected intradermally, the immune response skewed towards Th2 **(**Eusébio et AL, 2021).

Injection Dose: Studies have shown that there is no direct correlation between the injection dose of a DNA vaccine and the induced immunity, and using excessive amounts of DNA vaccine in each dose does not increase its efficacy but rather decreases immune system stimulation. Hence, determining the optimal dose of DNA vaccines is crucial (Eusébio et AL, 2021).

Advantages:

1. Antigen production by host cells: This mimics some aspects of inactivated virus vaccines without the risk of replicating pathogenic organisms (Rezaie et al, 2020).

2. No need for a cold chain: This reduces storage and production costs (Rezaie et al, 2020).

3. Stimulates both arms of the immune system: By presenting the carrier gene through both MHC class I and MHC class II pathways (Rezaie et al, 2020).

DNA vaccines can be considered subunit vaccines since they only express part of a protein. However, unlike subunit vaccines, DNA vaccines do not require production and purification in fermenters. During purification, the native form of the protein can be easily altered, and antibodies produced against this altered protein may not neutralize the main pathogen. Additionally, subunit vaccines are only presented by MHC class II, while DNA vaccines are presented by both MHC classes. DNA vaccines are stable at high and low temperatures and can be stored in a dry or solution form, eliminating the need for a cold chain, which accounts for 80% of vaccination costs for many vaccines. The uniform type of vaccine for all antigens also allows for easy administration of multiple vaccines simultaneously. Moreover, using a single method for producing all vaccines simplifies the production process. Faster DNA production compared to proteins or pathogens and simpler purification are additional advantages of these vaccines (Rezaie et al, 2020).

Disadvantages: Potential Integration of Plasmid DNA into Cell Genomes: Although theoretically possible, this occurrence is very rare. No such integration of plasmid DNA into host genomes has been reported to date (Rezaie et al, 2020).

Tolerance: In some experimental systems, repeated low doses of antigen injection can lead to immunological unresponsiveness or tolerance. In DNA vaccines, since only a small amount of antigen is expressed for an extended period, tolerance may occur (Rezaie et al, 2020).

Autoimmunity: There is a possibility of autoimmunity resulting from antigen expression on cell surfaces and immune reactions against the cell, or converting cells that do not naturally present antigen into antigen-presenting cells. However, it should be noted that viral or bacterial infections may also cause this, and the risk from DNA vaccines is not higher than from these infections (Rezaie et al, 2020).

Anti-DNA Antibodies: Inducing anti-plasmid antibodies is another concern discussed in DNA vaccines. Antibodies against DNA are produced in diseases like lupus. In the case of plasmids, the

likelihood of producing such antibodies is lower since purified double-stranded DNA may not easily stimulate antibody production. Although theoretically possible, none of these issues have been reported so far (Rezaie et al, 2020).

***Adenoviruses*:** These viruses have a high gene transfer and expression capacity in transgenic settings and can infect both dividing and non-dividing cells. One of the weaknesses of adenoviruses is that many people are resistant to them due to previous exposure. Commonly, human adenovirus serotype 5 is used to produce these vaccines because it can be easily produced in large quantities (Ura et al, 2024).As of April 2021, four adenovirus vector vaccines for COVID-19 had received authorization for use in at least one country:

- ✓ Oxford-AstraZeneca *COVID-19* Vaccine: This vaccine uses a modified chimpanzee adenovirus (ChAdOx1) (Chakraborty & Parvez, 2020).
- ✓ Sputnik V *COVID-19* Vaccine: This vaccine uses human adenovirus serotype 26 for the first dose and serotype 5 for the second dose (Chakraborty & Parvez, 2020).
- ✓ Johnson & Johnson *COVID-19* Vaccine: This vaccine uses serotype 26 (Chakraborty & Parvez, 2020).
- ✓ CanSino *COVID-19* Vaccine: This vaccine also uses serotype 5 (Jonathan, 2020).

The first dose of the Ebola virus vaccine (Zabdeno/Mvabea) uses human adenovirus serotype 26 expressing the Ebola virus glycoprotein from the Mayinga strain. The vector viruses used in both doses of this vaccine are replication-deficient and carry genetic codes for several Ebola virus proteins (Wang et al, 2017).

Gumboro Disease: Recombinant vaccines against Gumboro Disease, also known as Infectious Bursal Disease (IBD) or Gumboro, are a viral immunosuppressive disease in birds that cause damage to the bursa of Fabricius. The causative agent is a virus from the Birnaviridae family. In recent years, due to the emergence of very virulent strains of this virus, the need for new vaccines has increased as these viruses deplete maternal antibodies.vp2 is one of the capsid antigens of the virus. In a comparative study between recombinant vaccines, vpx, and vp2 pp, the recombinant vaccine containing vp2 protein (expressed in baculovirus) was more effective than other vaccines. The mortality rate and bursa atrophy were lowest in the group vaccinated with this vaccine.In another study, after adding CPG ODN adjuvant to the recombinant vp2 vaccine expressed in E. coli and comparing it with vaccines containing only vp2 and attenuated vaccines, the highest antibody titers were found in chickens that received vp2 with the adjuvant. CPG ODN adjuvant is a non-specific immune activator with lower toxicity than other adjuvants. Bursa atrophy was lowest in chickens vaccinated with vp2 along with the adjuvant.Another type of recombinant vaccine expressed in turkey herpesvirus (HVT), which actively expresses interleukin II, used simultaneously with low doses of IBD virus strain 689 vaccines (isolated from low-infection chickens) produced in eggs, showed no effect on chick hatching. No clinical symptoms were observed in the chicks after hatching, and these vaccines increased antibody titers in this group of chicks.The result of these comparisons is as follows:The VP2 component of the IBD virus is very suitable for creating recombinant vaccines as it provides very high protective immunity in chicks. Nevertheless, new recombinant vaccines must offer more benefits than classic vaccines to be accepted by the poultry industry (in terms of safety, effectiveness, production cost, etc.). One advantage of these recombinant vaccines is significantly increasing humoral and cellular immunity levels. Efforts to develop new recombinant vaccines for this disease in fowlpox virus, Marek's disease, etc., are ongoing (Wagari, 2021).

Infectious Laryngotracheitis Virus (ILT): Advantages of Using Recombinant Vaccines: Recombinant vaccines are utilized, for example, to control Marek's disease and infections like laryngotracheitis. The turkey *herpesvirus* (HVT) is used as a vector for expressing the infectious laryngotracheitis virus (ILT). ILT is an acute respiratory disease with high infection rates caused by a herpesvirus that affects all ages of chickens. This disease is commercially significant in the poultry industry due to substantial losses in meat and egg production(Abdelaziz et al, 2024). Conventional vaccines against ILT are based on modified live viruses. They are highly effective but often identified with several adverse effects, such as the spread of vaccine strains to non-vaccinated groups, leading to increased virulence and the existence of latent carriers, ultimately causing severe virus outbreaks in farms. On the other hand, HVT-based vaccines are intended to prevent Marek's disease .Immune Performance: As an example, the use of developed recombinant vaccines to improve Marek's disease and ILT was examined. The vaccine contents result in the expression of two ILT virus glycoproteins. These glycoproteins are essential for initiating infectious processes and developing immune response and protection against infections. Birds subcutaneously vaccinated with recombinant ILT vaccines and unvaccinated birds were challenged, and observations at various ages were examined to measure the presence of ILT clinical symptoms (Hein et al, 2021).

***Human Papillomavirus* (HPV):**Another example of a recombinant protein vaccine is the *human papillomavirus* (HPV) vaccine. Currently, there are two vaccines against HPV infection, both based on virus-like particles (VLPs) assembled from recombinant HPV coat proteins. Prokaryotic expression systems for vaccine antigen production include bacteria such as *E. coli*, and eukaryotic systems include mammalian, yeast, or insect cells. Several factors are considered before selecting an appropriate system for antigen expression, including expression levels, selection markers, and the presence or absence of post-translational modifications, all of which are crucial for the effective production of recombinant antigens as vaccines. Bacterial expression systems are widely used due to their ease of use and high capacity for expression. However, for antigens requiring post-translational modifications, mammalian, yeast, or insect cells are considered. Although recombinant protein-based vaccines offer numerous advantages such as safety and production economy, most of them are weak in eliciting a durable immune response on their own and thus require the use of adjuvants to create a more lasting immune response (Romano & Zanetti, 2022).

Recombinant Antifungal Vaccines: Overall, extensive research efforts have been made over the years to develop vaccines for opportunistic and endemic fungal infections in humans and animals. Several comprehensive reviews and interpretations are available. To date, most work has focused on vaccines for human infections caused by Candida species. However, significant efforts have also been made to develop vaccines for cryptococcosis, coccidioidomycosis, blastomycosis, histoplasmosis, paracoccidioidomycosis, infections caused by Pneumocystis, and more recently, aspergillosis. Despite extensive research on these vaccines, none have been approved by the U.S. Food and Drug Administration (FDA) for active or passive immunization in humans (Nisbet et al, 2013).

Fungal Cell Wall Structure: Fungi and animals are phylogenetically grouped within the same eukaryotic domain, so it is not surprising that there are many similarities between fungal and human cells. These are important considerations for drug discovery and treatment of fungal diseases. A major difference between the fungi kingdom and animals is the presence of a cell wall on almost all fungal cells. As a result, proteins that synthesize and remodel the cell wall are important drug targets. Additionally, fungal cell wall components are recognized by the innate immune system in humans, leading to adaptive and trained immune responses. The cell wall is primarily composed of cross-linked carbohydrate polymers and mannoproteins, recognized by pattern recognition receptors on host immune cells, especially monocytes, macrophages, and dendritic cells. The most abundant components of the fungal cell wall are mannoproteins and beta-glucans, followed by chitin/chitosan. Mannoproteins are mainly found on the outer part of the cell wall, beta-glucans in the middle, and

chitin/chitosan towards the inner part of the cell wall. These cell wall components are virtually found in all invasive fungal pathogens. Some fungal cell walls also contain galactomannan, α-glucan, and melanin (Romano & Zanetti, 2022). Additionally, the presence of a polysaccharide capsule on Cryptococcus species, mainly composed of glucuronoxylomannan (GXM) and galactoxylomannan, is notable. The capsule is a dominant virulence factor(Romano and Zanetti, 2022). Structurally, the capsule covers the cell wall and among its various roles, it "masks" the recognition of cell wall ligands by pattern recognition receptors, thus interfering with immune development. Finally, fungi release extracellular vesicles containing proteins, lipids, and nucleic acids. Besides secretion into the extracellular environment, extracellular vesicles may shuttle to the cell wall, aiding in cell wall remodeling and surface protein expression. Host encounters dynamic changes in the distribution and amount of fungal cell wall components associated with morphological changes during infection. External stress events, transition between yeast and hyphal growth, and the cell division process can impact the cell wall to reduce host recognition, disrupt inflammatory responses, and increase fungal pathogenicity (Oliveira et al, 2021).

Classification of Fungal Vaccines and Adjuvants: Fungal vaccines can be broadly categorized based on their components, ranging from multiple antigens to single antigens:

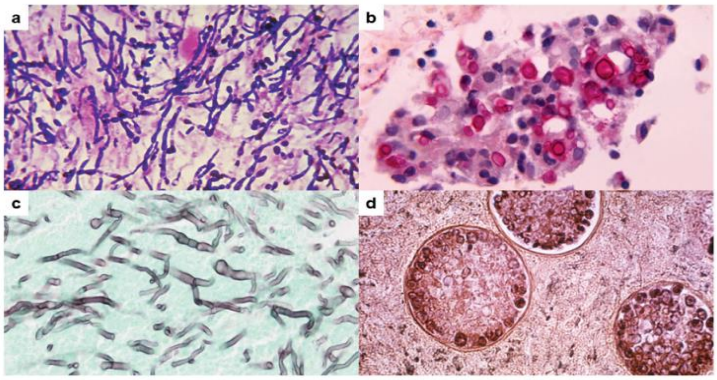
1. Whole organism vaccines (live attenuated or killed fungal cells)
2. Crude extracts (fractions derived from fungal cells and culture media)
3. Purified subunit vaccines (proteins, peptides)
4. Nucleic acids (RNA and DNA) that encode the desired antigen(s) (Table 1) (Oliveira et al, 2021).

Table 1: Overview of the Advantages and Disadvantages of Major Fungal Vaccine Categories (Oliveira et al, 2021).

Row	Vaccine Category	Advantages	Disadvantages
1	Live attenuated	Strong, long-lasting, immunogenic, simple production processes.	Risk of persistent infection in immunocompromised populations; potential for autoimmunity.
2	Killed fungi	Cannot cause infection; more stable compared to live attenuated vaccines; simple production processes.	Elicit less robust immune responses compared to live vaccines; potential for autoimmunity.
3	Fungal extract	Contains multiple multivalent antigens.	Reactogenicity; autoimmunity.
4	Purified proteins, peptides, carbohydrates, and lipids	Fewer antigens minimize potential side effects.	Narrow immune response due to fewer antigens; adjuvant needed. Precise epitope selection, antigen design, and purification required.
5	Nucleic acids (RNA and DNA)	Rapid production process; strong immune responses.	Risk of unwanted immune reactions; strict temperature conditions for storage.

Potential Fungal Vaccines: Candida, Cryptococcus, Aspergillus, and Pneumocystis are the most common fungal genera causing invasive infections in humans. Endemic dimorphic fungi such as Histoplasma, Coccidioides, Paracoccidioides, and Blastomyces also cause invasive mycoses. Examples of different fungal morphologies in human tissue are shown in Figure 2. Other fungi causing serious infections include species of Mucor, Sporothrix, Scedosporium, and Fusarium (Oliveira et al, 2021).

Figure 2: Examples of Fungal Morphological Diversity in Human Tissue from Patients with FungalInfections(Oliveira et al, 2021).



Challenges of Fungal Vaccines: Need to Define Target Population: Many invasive fungi tend to cause disease in immunocompromised individuals. Specific knowledge of the type of protective response needed to combat a particular fungus is required, and then there is a need to translate this information into a formulation that remains safe and effective in an immunocompromised host. Complexity of Fungal Cells: Fungi are eukaryotic, and pathogenic species have distinct differences and similarities with human cells. Fungal cells have a dual protective layer: an inner plasma membrane and an outer cell wall. The plasma membrane is a phospholipid bilayer that may vary in composition due to the presence of specific fungal sterols in different species. Ergosterol, similar to human cholesterol, is particularly important for membrane fluidity and essential for its survival (Silva et al, 2020). Lack of High-Quality Vaccines ‹ Formulation ‹Lack of Appeal in the Mass Market (Cassone & Casadevall, 2012).

***Candida*:** *Candida* species usually colonize humans as commensal organisms. However, under conditions of immune suppression, they can opportunistically become pathogens. *Candida albicans* and *non-albicans* species are the most common cause of life-threatening invasive fungal infections. Globally, it is estimated that 700,000 people suffer from invasive candidiasis annually, with associated mortality rates potentially exceeding 50%. In addition, Candida can cause the following infections:

1. Skin infections

2. Mucosal infections, such as vulvovaginal candidiasis, which, although rarely fatal, is associated with significant morbidity.

3. Disseminated(Oliveira et al, 2021).

For example, vulvovaginal candidiasis can profoundly negatively impact the quality of life. Most

women are estimated to experience vulvovaginal candidiasis at least once in their lifetime, and many suffer from recurrent disease. Most importantly, the emergence of drug-resistant strains, such as C. auris, is a public health concern. Therefore, the need for new treatments and protective vaccines against *Candida* has increased. Using mouse models of vulvovaginal and/or systemic candidiasis, the effectiveness of anti-Candida vaccines in targeting Candida's virulence factors and pathogenic forms, such as hyphae and cell wall antigens, has been demonstrated with various formulations, including live attenuated vaccines (in general). However, morphological, phenotypic, and genetic diversity among *Candida* species poses a challenge for vaccine development. Furthermore, since *Candida* is a human commensal, there is a theoretical concern that Candida vaccines could disrupt the natural microbiota. Targeting specific antigens in the invasive hyphal form could minimize these potential issues. From the host's side, diverse infection sites and various types of immune deficiencies in at-risk groups present barriers to designing a vaccine that broadly protects against this wide clinical spectrum of disease(Oliveira et al, 2021).

Vaccine for *Candida*:Recent research has led to two recombinant Candida vaccines reaching human clinical trials with promising results:

1. PEV7
2. NDV-3

PEV7 and NDV-3A vaccines are each formulated with a protein antigen. Given the intra- and inter-species antigenic diversity, including differences between yeast and hyphal forms, many researchers aim to develop multivalent Candida vaccines offering broader protection. This tactic has the potential to expand the immune response's protective scope while reducing Candida's ability to evade host immunity. Approaches include combining known immunogenic antigens and using in silico analysis to identify dominant Candida antigens for the immune system (Oliveira et al, 2021).

***Cryptococcus*:** *Cryptococcus neoformans* and *C. gattii* species complexes cause cryptococcosis. Infection is thought to often start after pulmonary inhalation. Subsequent dissemination to other organ systems, particularly the central nervous system, can occur. Individuals with compromised T-cell immunity are particularly susceptible. It is estimated that over 220,000 cases of cryptococcal meningitis occur annually among HIV-infected individuals globally, resulting in approximately 180,000 deaths. Cryptococcus is unique among medically significant fungi and presents opportunities and challenges for vaccine development (Oliveira et al, 2021).

Vaccine for *Cryptococcus*: The capsule of this fungus is primarily composed of two large polysaccharides, glucuronoxylomannan (GXM) and glucuronoxylomannogalactan (GXMGal), with poor immunogenicity. To improve the GXM antigen, a vaccine containing GXM conjugated to tetanus toxin was developed. Immunized mice produced antibodies against GXM and were partially protected following a challenge with C. neoformans. Similarly, a GXM-mimicking peptide conjugate generated protective antibodies. This vaccine induced a protective Th1-type adaptive immune response and was effective even when heat-killed. Mice were also protected from lethal challenges with mutated vaccine strains of *C. neoformans*: 1) overexpressing the transcription factor Znf2, 2) lacking sterylglucosidase, resulting in the accumulation of glycolipid sterylglucoside in the cell membrane, 3) lacking the F-box protein Fbp1 (Oliveira et al, 2021).

***Aspergillus*:** *Aspergillus* is a filamentous fungus found worldwide, commonly in soil and decaying vegetation. Airborne spores (conidia) are regularly inhaled and typically controlled by host defenses without causing harm. However, in immunocompromised individuals, conidial

germination into invasive tissue hyphae can cause a range of diseases from acute to chronic. Those at higher risk include individuals with neutropenia, stem cell and solid organ transplant recipients, and those receiving immunosuppressive therapy such as corticosteroids. Essentially, these conditions challenge vaccine development for this target population, as protective immunity may be reduced, along with the need for the host to recognize and combat various fungal structures like conidia, microtubules, and hyphae. *A. fumigatus* is the most common *Aspergillus* species responsible for human opportunistic infections, although other species, typically *A. flavus, A. niger, A. terreus,* and *A. nidulans,* can also cause disease. Invasive aspergillosis is responsible for over 200,000 cases annually and is associated with high mortality rates. Additionally, allergic manifestations can occur with sensitization to *Aspergillus* allergens, typically in patients with cystic fibrosis, severe asthma with fungal sensitivity, and allergic bronchopulmonary aspergillosis, the latter estimated to affect around 5 million individuals. Therapeutic vaccines to reduce allergic responses and immune skewing towards protective responses hold promise for these challenging diseases but are still in the early stages of development. Another potential market for Aspergillus vaccines targets avian aspergillosis. Disease outbreaks in commercial poultry flocks, particularly turkeys, can have major economic implications (Oliveira et al, 2021). However, despite several laboratories investigating the development of a safe and effective vaccine against aspergillosis and obtaining promising results in experimental models using homologous proteins, crude extracts, or recombinant allergens from Aspergillus, no vaccine currently exists for aspergillosis (Silva et al, 2020).

***Coccidioidomycosis*:** *Coccidioides* spp. are environmental pathogens responsible for the respiratory disease coccidioidomycosis in humans, primarily occurring in desert soils in the southwestern United States and parts of Mexico and Central and South America. Recent studies also indicate that 17 to 29 percent of pneumonia cases in endemic areas are due to coccidioidomycosis, and these endemic areas are expanding. Pursuing a vaccine for coccidioidomycosis is not a new idea. In fact, a formalin-killed spherule vaccine was developed, and a clinical trial was conducted. Following the outcome with the whole-cell vaccine, researchers have shifted their attention to specific antigens for study and development (Silva et al, 2020).

Immunotherapy: Generally, fungal immunotherapy involves administering exogenous immune factors such as white blood cells, antibodies, and cytokines to beneficially alter the course of infection. Monoclonal antibodies (mAbs) and dendritic cell (DC) therapy and vaccine strategies have been explored to treat or prevent fungal infections (Santos & Levitz, 2014).

Immunotherapy and Dendritic Cell Vaccination:DC immunotherapy involves incubating or "pulsing" DCs ex vivo with selected antigens or pathogens and then reintroducing the cells into the host to boost protection against an infectious agent. This therapeutic strategy can be compared to DC vaccination, where the goal is to protect the host against future pathogen exposure. While the technical process is identical, the main difference between the two strategies lies in the timing of intervention. DC vaccination requires treatment before infection, whereas DC immunotherapy is administered post-diagnosis (Santos & Levitz, 2014).

Antibody Therapy: Antibodies or immunoglobulins recognize diverse antigens through genetic rearrangement and somatic hypermutation of their variable regions. The constant regions, defined by immunoglobulin isotypes, are recognized by Fc receptors on immune cells and C1q, a factor involved in the complement cascade, leading to bacterial but not fungal lysis. Fungi resist lysis with their rigid cell wall composed of a fibrous polysaccharide skeleton (Santos & Levitz, 2014).

Recombinant Antibacterial Vaccine: Using live bacterial cells as vehicles for delivering recombinant antigens has emerged as an intriguing alternative for developing new vaccines over

the past two decades. The evolution of genetic engineering techniques has enabled the construction of recombinant microorganisms capable of expressing heterologous proteins in various cellular compartments, enhancing their potential as delivery systems for vaccines against viruses, bacteria, and parasites. The inherent properties of these microorganisms, such as lipopolysaccharides in Gram-negative bacteria or lipoteichoic acid in Gram-positive bacteria, along with other pathogen-associated molecular patterns (PAMPs), are recognized by pattern recognition receptors (PRRs), mediating various signaling pathways that lead to the production of inflammatory cytokines and the expression of other antimicrobial genes. This innate immune response to bacterial pathogens and its effect on the adaptive immune system make live attenuated microorganisms highly efficient vehicles for stimulating specific and long-lasting immune responses against the carried antigens. Thus, in addition to producing and delivering antigens, the intrinsic properties of these carriers can also enable them to act as beneficial immune-stimulating adjuvants .(Adilson José da Silva et al, 2015).Recombinant vaccines can be created through various methods. These vaccines are produced using expression systems such as bacteria, insects, yeast, plants, mammals, and even cell-free systems (Cox, 2021).

Advantages and Disadvantages: Each type of expression system has several advantages and disadvantages. Bacterial expression, typically using E. coli, is the most popular organism for recombinant protein expression. Its advantages include:

1. Ease of bioprocessing
2. Capability for large-scale production
3. Easier strain engineering
4. Lower cost
5. Shorter production times
6. Genetic combination
7. Ease of culture and scalability
8. Rapid expression
9. High yield
10. However, there are disadvantages to using E. coli for expression, which include:
11. Lack of an efficient post-translational modification machinery
12. Difficulty in producing high molecular weight proteins

Insoluble expression (one of the major drawbacks): This phenomenon occurs when overexpression leads to the formation of insoluble aggregates (inclusion bodies). To overcome this issue, processes such as protein refolding and optimization at the molecular level in the lab can be carried out to achieve desirable results. This can be done by optimizing expression conditions, such as testing different strains or adjusting growth and induction conditions, changing media, buffers, and co-expressing chaperones, and in some cases, even improving cell lysis conditions (Cox, 2021).

Treatment: Treating microbial infections requires the use of antibiotics. Research has shown

that increased antibiotic use leads to the rise of antibiotic-resistant strains, while studies show that infections caused by these bacteria increase infection-related complications, mortality, and treatment costs. One method to reduce antibiotic use is through vaccines. Nowadays, live recombinant microorganism-based vaccines are used as "biologics" and administered orally to prevent or treat certain diseases (Aliramaei et al, 2020).

***Brucella*:** The bacterium Brucella is an obligate intracellular pathogen causing brucellosis in humans and animals and is highly contagious. The common vaccine for combating this pathogen typically uses the whole bacterial cell, leading to numerous side effects. Nowadays, there is significant interest in using subunit vaccines to eliminate pathogenic parts and enhance immunogenic parts (Nazifi, 2024). Brucellosis is a treatment-resistant disease with a long treatment duration and various economic and psychological problems for society and individuals. What distinguishes brucellosis in Iran from other countries is its endemic nature. This disease is present in all seasons but is more prevalent in spring and summer, the birthing and lactation seasons for livestock (Nazifi, 2024).Prevention through vaccination is the most effective way to reduce disease incidence. Researchers' interest in developing new and updated vaccination techniques has led to the development of new generations of safer vaccines, as this category of vaccines uses immunizing components of pathogens(Jaydari et al, 2020). Moreover, using recombinant DNA-based techniques has led to the production of third-generation vaccines that extend beyond the second generation of vaccines, which included effective immunogenic components of microorganisms, adding to the first generation of vaccines that used weakened and inactivated pathogens. The formulation and characteristics of these vaccines are continuously reviewed by researchers and pharmaceutical manufacturers. Furthermore, engineered Gram-positive and Gram-negative bacteria can be used as platforms for producing and expressing immunological proteins in these vaccines (Nazifi, 2024).

***Listeria monocytogenes*:** Infection with *Listeria monocytogenes* (listeriosis) is a rare but preventable foodborne disease that can cause bacteremia, meningitis, fetal loss, and death, with increased risk for the elderly, pregnant women, and individuals with conditions that weaken the immune system. Attenuation of *Listeria monocytogenes* for vaccine purposes has been achieved using auxotrophic mutations or deletion of virulence factors such as actA and inlB genes.(Adilson José da Silva et al, 2015).

***Bacillus subtilis*:** Bacterial spores serve as vaccine carriers. Recent research with *Bacillus subtilis* has shown the ability to deliver antigens and induce immune responses using bacterial spores as carriers. Despite poor immunogenicity due to low antigen expression levels in spores and their short residence time in the host gastrointestinal tract after oral vaccination, their greater resistance to adverse conditions over extended periods, thermal resistance, probiotic effects, low production costs, and GRAS (Generally Recognized As Safe) status make B. subtilis spores attractive for vaccine antigen production (Adilson José da Silva et al, 2015).

***Botulinum Neurotoxin* Type *E* Vaccine:** Intracutaneous vaccination with the DNA vaccine for *botulinum neurotoxin serotype E* induces cellular and humoral immunity and protects against lethal toxin challenges. *Botulinum neurotoxins* (BoNTs), produced by spore-forming, Gram-positive, anaerobic *Clostridium botulinum* bacteria, are the most toxic substances known, causing botulism, flaccid paralysis, or death. Due to their high lethality, the Centers for Disease Control and Prevention (CDC) classify BoNTs as Category A agents. Currently, no vaccine exists to protect against BoNTs, making the rapid production of a safe and effective vaccine crucial. DNA-based vaccines have recently attracted significant attention as they can be quickly produced and utilized in mass vaccination strategies to prevent disease outbreaks (Romano and Zanetti, 2022).

The Immunogenic and Protective Effect of a DNA Vaccine: We encode a 50 kDa carboxy-

terminal fragment of the BoNT/E heavy chain delivered via an intracutaneous route. This plasmid DNA vaccine has induced BoNT/E-specific humoral and cellular immune responses and provided full protection to animals against lethal challenges with BoNT/E. These results not only suggest that DNA vaccines can be developed as safe and effective candidates against BoNTs but also propose a potential method for producing vaccines that protect against biothreat toxins(Romano & Zanetti, 2022).

***Lactococcus lactis*:** *Lactococcus lactis* is part of the *lactic acid bacteria* group, Gram-positive, facultatively anaerobic, non-sporulating, and non-motile. Live *lactic acid bacteria*, particularly *Lactococcus lactis*, are non-pathogenic, non-invasive, and safe. Studies have been conducted on the benefits and limitations of recombinant *Lactococcus lactis*-based vaccines, focusing on oral vaccines, methods for vaccine enhancement, and future prospects as a promising strategy for vaccine production and preventing certain infectious diseases. Vaccines used for preventing and controlling pathogens include DNA vaccines, subunit vaccines, live attenuated vaccines, and vector-based vaccines. Two main types of live bacterial carriers include non-pathogenic live bacteria and live attenuated pathogenic bacteria (Aliramaei et al, 2020).

Features and Applications of *Lactococcus lactis*:

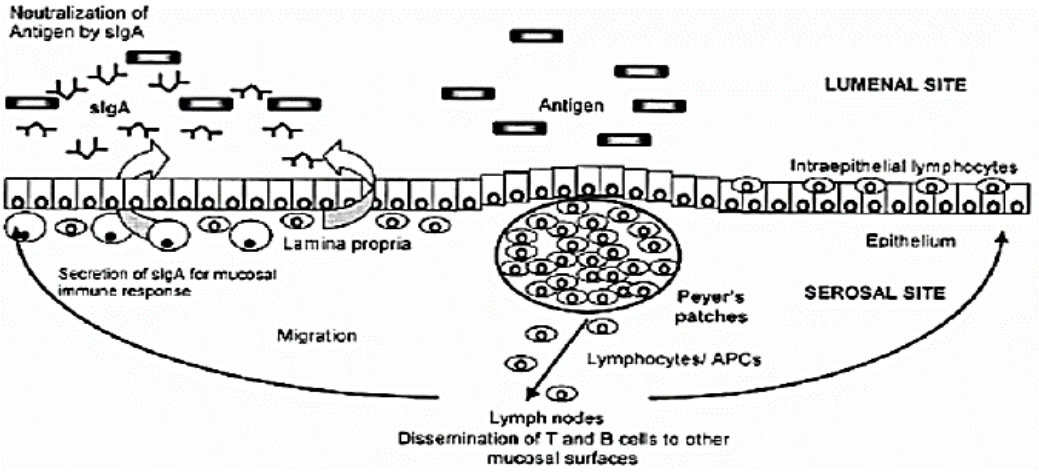
1. Economic significance
2. Complete genome sequencing
3. Easy genetic manipulation
4. Development and expansion of various genetic tools
5. Simple and uncomplicated metabolic pathway
6. Energy acquisition by converting sugars to pyruvate via glycolytic pathway and substrate-level phosphorylation
7. Best member of *LAB*
8. Model microorganism used in food products
9. Commercial and cost-effective production of important proteins in fermenters
10. Production of certain food products containing recombinant proteins
11. As an antigen carrier in vaccination
12. No colonization in the gastrointestinal tract
13. Tolerance to acidic conditions (survival during passage through the stomach)
14. High stability in the presence of bile salts
15. Increased production of heat shock protein chaperones GroEL and GroES for better solvent and higher temperature tolerance
16. No lipopolysaccharides (endotoxin) in their cell structure (Aliramaei et al, 2020).

Immunization After Oral Consumption of Recombinant *Lactococcus lactis*: Given that mucosal

surfaces are the entry point for many pathogens, inducing specific mucosal immunity, such as secretory IgA antibodies, through mucosal vaccination methods (oral, intranasal, vaginal, and rectal) can help eliminate infections early and efficiently. Additionally, mucosal vaccination has the ability to induce both systemic and mucosal immune responses. During oral consumption of *Lactococcus lactis* bacteria, due to its non-commensal nature and non-colonization in the gastrointestinal tract, vaccine delivery to the M cells of the intestinal mucosa is similar to microparticle vaccine delivery systems (Aliramaei et al, 2020).

Induction of Mucosal Immune Response Using Oral Vaccine: As illustrated in Figure 3, during oral administration of this vaccine, bacteria are taken up by M cells in the Peyer's patches, cross the epithelium, and reach antigen-presenting cells (mostly dendritic cells). Processed antigens are presented by antigen-presenting cells, triggering an immune response (Aliramaei et al, 2020).

Figure 3: Induction of Mucosal Immune Response Using Oral Vaccine



Recombinant Antiparasitic Vaccines: *Nematode* infections in livestock and humans have devastating effects on health and productivity, affecting food security worldwide. Despite decades of research, developing recombinant vaccines against these pathogens has been unsuccessful. The primary cause of parasitic gastroenteritis (PGE) in small ruminants in temperate regions worldwide is the nematode *Teladorsagia circumcincta*. Infection occurs through ingestion of third-stage larvae from pasture. The immature and mature worms then reside in the host's abomasum, causing significant production losses. Currently, *T. circumcincta* is controlled using anthelmintics. However, widespread multi-drug resistance makes developing a vaccine a priority. Protective immunity against *T. circumcincta* develops after prolonged and consistent exposure, with the degree of immunity dependent on the parasite challenge level, animal age, and genotype. Immunity is associated with reduced nematode larval establishment and growth rates in the abomasal glands and decreased egg output from adults. The mechanisms responsible for these effects are complex: immediate hypersensitivity reactions, cellular factors, and humoral responses have all been demonstrated. Studies on immunizing sheep with a complex of eight recombinant proteins have achieved protective levels against T. circumcincta higher than those observed in any other system using recombinant vaccines against a parasitic nematode in a definitive ruminant host (Nisbet et al, 2013).

Types of Antiparasitic Vaccines:

1. Protozoan Vaccines: In the case of pathogenic protozoa, attenuated strains are used for inoculation, successfully stimulating protective immunity in animals. Attenuated strains used for protozoa inoculation include:

2. Poultry vaccines against Eimeria: Using precocious strains as vaccines.

3. Protozoan strains: Altered life cycles that do not form tissue cysts, such as Toxoplasma, which has reduced virulence due to repeated passage and is used as a vaccine to prevent abortion in sheep.

4. Babesia: In animals whose spleens have been removed, reduced virulence strains produce Babesia vaccine strains used against Babesia bigemina and Babesia bovis.

5. Giardia Vaccines: Developed for dogs and cats, vaccinated dogs exhibit mild clinical symptoms and do not develop Giardia cysts when infected with virulent Giardia strains. Such vaccines are also commercially available.

6. Leishmaniasis Vaccine: Against Leishmania infantum in dogs using Leishmania infantum fucose and mannose ligand.

7. Recombinant Coccidiosis Vaccine: Developed using Eimeria maxima gametocyte antigens and made available (Garedaghi, 2024).

4. Conclusion:

Vaccines are one of the most cost-effective measures available for protecting against infectious diseases, including fungal, viral, parasitic, and bacterial infections For instance, research by Oliveira et al. in 2021 highlighted the role of vaccines in eradicating smallpox and significantly reducing polio, measles, diphtheria, and pneumococcal infections. Additionally, according to the World Health Organization (WHO), vaccines have been successfully used against more than 25 debilitating or life-threatening diseases, including tetanus, rabies, influenza, meningitis, cholera, rubella, and hepatitis B (Oliveira et al, 2021).Significant progress has been made in developing fungal vaccines for human use. Animal studies have shown protection against all major medically significant fungi using various vaccine designs, from subunit formulations to live attenuated fungi. Superior efficacy has been demonstrated using new adjuvants and delivery systems aimed at stimulating immune system arms crucial for controlling fungal invasions. Three vaccines have entered human trials, showing the potential for conducting clinical trials targeting at-risk populations (Oliveira et al, 2021). Using genetically modified organisms (GMOs) raises concerns, such as the risk of horizontal gene transfer to other organisms in the host's mucosal tissue, the impact of the product expressed by the delivered or replaced genes, and the use of antibiotic resistance genes as selection markers. Aliramaei et al. in 2020 emphasized the need for developing suitable strategies in future clinical programs to limit environmental contamination when using Lactococcus lactis as a live vaccine (Aliramaei et al, 2020). In 2024, Garedaghi reported that a limited number of protozoan vaccines, such as Theileria, Babesia, coccidial, toxoplasmic, and Leishmania vaccines, have been developed and produced as attenuated vaccines. These vaccines come with challenges and obstacles. For molecular parasitic vaccines, high reproductive safety vaccines have not yet been developed, and research continues. Currently, only anti-tick vaccines like Tick Guard in Australia and Gavac in Cuba are used in South American countries, with success rates of 30-35%. Further studies are necessary to develop molecular anti-tick vaccines with efficiencies above 35% (Garedaghi, 2024). Vaccines are among the greatest achievements in medicine (23). Despite significant progress in vaccine discovery, continuous research is essential for maintaining vaccine development and timely commercial availability. Further advances in vaccine discovery, development, and formulation should pave the way for highly safer and more efficient vaccines, restoring public confidence in vaccines and mass vaccination programs as a key public health strategy during pandemics (Veysel،2021).

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