The Role of Recombinant DNA Technology in Vaccine Development

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Abstract: Recombinant DNA is created by combining DNA sequences that would not normally occur together in nature and it is first incorporated in vector or plasmid. Vectors are DNA molecules that are capable of replicating in the host cells and act as a carrier molecule for the construction of recombinant DNA. The development of rDNA procedures has provided some unique opportunities for vaccine production, which are tend to be more stable, effective and safe. Development of vaccination as a tool in fighting disease has resulted in the potential to combat almost all infectious agents affecting people and animals. Current public health threats posed by the potential spread of highly infectious disease agents between animals and humans, as well as the emergence of new diseases, impact animal agriculture significantly. Animal vaccinations are among the most effective, successful tools for dealing with these concerns. However, the uses of rDNA vaccines have a great importance, there are still limitations for developing that are requiring a great attention.
[Worku A, Mebratu A, Yitayew D, Dagmawi Y, Ayalew N and Kalkidan G. **The Role of Recombinant DNA Technology in Vaccine Development.** *Nat Sci* 2016;14(11):8-16]. ISSN 1545-0740 (print); ISSN 2375-7167 (online). http://www.sciencepub.net/nature. 2. doi:10.7537/marsnsj141116.02.

Key words: *Development, Recombinant DNA, Vaccines, Vectors.*

1. Introduction

Genetic engineering refers to a group of techniques used for genetic modification of organisms. These procedures are of use to identify, replicate, modify and transfer the genetic material of cells, tissues or complete organisms (Izquierdo, 2001; Karp, 2002). The techniques are generally related to the direct manipulation of DNA oriented to the expression of particular genes (Hugon, 2006).

Recombinant DNA (rDNA) is defined as a DNA sequence artificially obtained by combining genetic material from different organisms, as is the case for a plasmid containing gene of interest (Rossana and Cristina, 2010). Recombination is Exchange of genetic information between chromosomes or other molecules of DNA which is divided into homologous and non-homologous (Clark, 2005).

The term vaccine (from the Latin term "vacca" meaning cow) first coined by Edward Jenner to describe the inoculation of humans with cow pox virus to confer protection against the related human small pox virus and illustrates the close relationship between human and animal infectious sciences (Meeusa *et al*., 2007). Prevention of disease is the most desirable, most convenient and highly effective approach to health; this is achieved by immunization using biological preparation called vaccination (Sasidhara, 2006).Veterinary vaccines are preparations containing antigenic substances which are administered for the purpose of inducing specific and active immunity against disease provoked by bacteria, viruses, or other microorganisms like parasites, antigenic fractions or substances produced by these organisms (DACA, 2006).

Using recombinant deoxyribonucleic acid (rDNA) technologies, scientists have been able to develop many types of recombinant vaccines; that are classified as plant based sub-unit vaccines, genetic vaccines, recombinant inactivated vaccines, and live genetically modified vaccines; which are designed to be safer, more efficacious, and/or less expensive than traditional vaccines (Mark *et al*, 2008).

However, the knowledge, immunization process and application of recombinant DNA vaccines are yet limited. The information's available regarding rDNA technology is less and the technology is not used in Ethiopia. The need to provide the current advances in this area, the future perspective of the technology and personal interest to deal in this area are some of the justification and the driving forces to prepare this seminar paper.

Therefore, based on the above justifications this seminar paper comprises the following objectives;

- ❖ To pinpoint understanding about recombinant DNA technology.
- ❖ To give overview about vaccine development using recombinant DNA technology and to describe four basic categories of newer recombinant vaccines.

2. Overview Of The Techniques

Techniques in genetic engineering include; the isolation, cutting and transfer of specific DNA pieces, corresponding to specific genes (Lewin, 1999; Klug and Cummings, 2002).

2.1. Vectors

Vectors are DNA molecules used to transfer a gene into a host (microbial, plant, animal) cell; and to provide control elements for replication, selection and expression (Dominic, 2006). Artificial vectors are constructed by cutting and joining DNA molecules from different sources using various restriction endonucleases and DNA ligase (Anil and Neha, 2005). The minimal features of a vector consist of origin of replication, a selection gene (usually an antibiotic resistant gene), and a cloning site to introduce foreign DNA (Cornel, 2007).

2.1.1. Yeast artificial chromosomes

A yeast artificial chromosome (YAC) is a vector used to clone DNA fragments larger than 100 kb and up to 3000kb. A YAC is an artificially constructed chromosome that contains a centromere, telomeres and an autonomous replicating sequence (ARS) element required for replication and preservation in yeast cells. ARS elements are thought to act as replication origin (Strachan, 2011).

A YAC is built using an initial circular plasmid, which is typically broken into two linear molecules using restriction enzymes. DNA ligase is used to ligate a sequence or gene of interest between two linear molecules, forming a single large linear piece of DNA (Strachan, 2011).

YACs are capable of replicating and being selected in common bacterial hosts such as *Escherichia coli*, as well as in the budding yeast *Saccharomyces cerevisiae*. They are of relatively small size (approximately 12 kb) and of circular form when amplified or manipulated in *E. coli*, but rendered linear and of very large size, that is, several hundreds of kilo bases (kb), when introduced as cloning vectors in yeast. Their capacity to accept large DNA inserts enables them to reach the minimum size (150 kb) required for chromosome-like stability and for fidelity of transmission in yeast cells (Carlo and Kresimir, 2002).

2.1.2. Bacterial artificial chromosomes

Bacterial artificial chromosomes (BACs) are vectors that are made up of fragments up to 300 kb long. They are based on fertility (F) factors, the naturally occurring sex factor plasmid of *E.coli* (Cornel, 2007). BACs were first developed as a large insert cloning systems to facilitate the construction of DNA libraries to analyze genomic structure (Shizuya *et al.,* 1992). BACs can clone large DNA molecules, ranging from 150-700 kb, and averaging 350 kb. Another advantage of BAC over other vectors is its stability in cell culture and ease of manipulation. Because some recombinant viruses are too large to be generated by traditional techniques, BAC technology was developed to carry out genetic and functional studies of viruses, especially herpes virus (Warden *et al.,* 2011).

2.1.3. Plasmids

A plasmid is a small DNA molecule that is physically separate from, and can replicate independent of chromosomal DNA within a cell (Figure 1). It is most commonly found as small circular, double-stranded DNA molecule in bacteria, but sometimes present in archaea and eukaryotic organisms. Plasmids carry genes that may benefit survival of the organism (e.g. antibiotic resistance), and can frequently be transmitted from one bacterium to another (even of another species) via horizontal gene transfer. Artificial plasmids are widely used as vectors in molecular cloning, serving to drive the replication of recombinant DNA sequences within host organisms. Plasmid sizes vary from 1 to over 1,000 kilo bases (kb) (Lederberg, 1952).

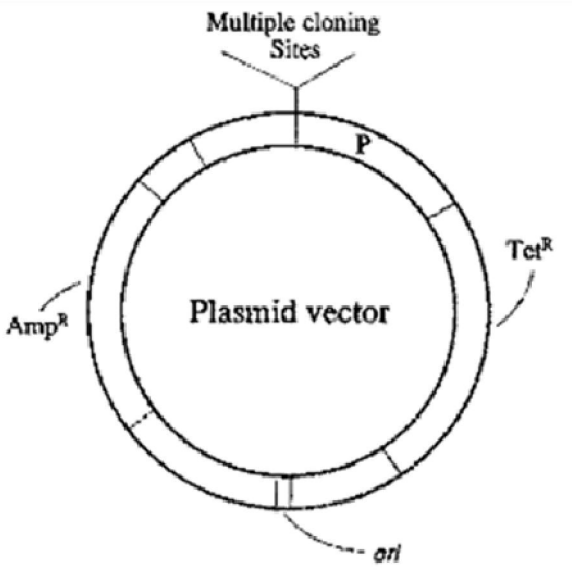

Figure 1: Structural organization of a plasmid vector. **Source:** (Dominic, 2006)

2.1.4. Phages

Bacteriophages are viruses that infect bacterial cells (Figure 2). A phage particle has a head and tail structure, consisting of a core of DNA within a protein coat (capsid) that is joined to a helical protein structure (Dominic, 2006). The most important phage in molecular biology is phage lambda, a phage with a 48.5-kb, linear, double-stranded DNA genome, packaged in a protein envelope. This phage infects *E. coli* and replicates in it (Cornel, 2007).

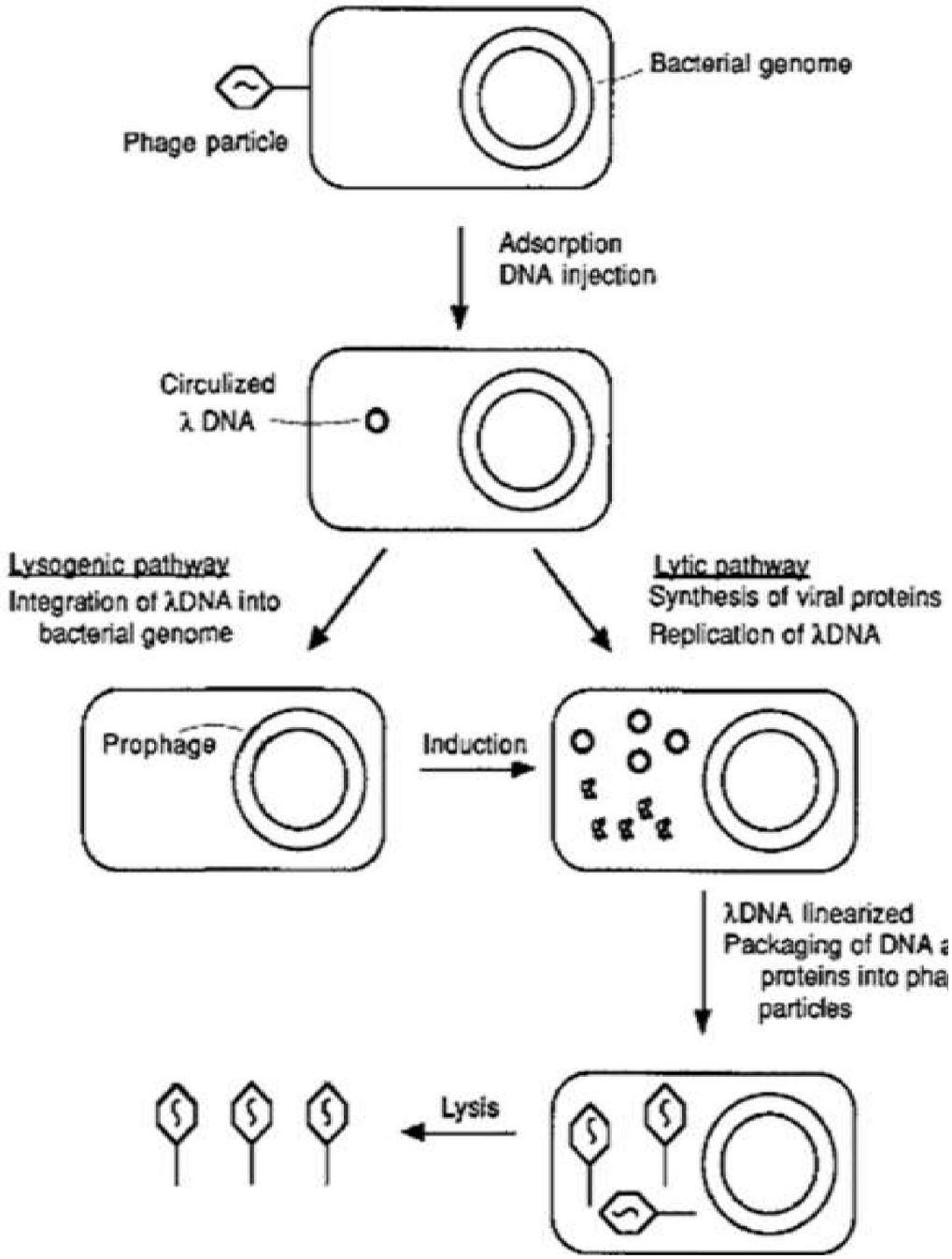

Figure 2: Life cycle of bacteriophage. **Source:** Dominic (2006).

2.1.5. Blue script plasmid

The pBluescript II phagemids (plasmids with a phage origin) are cloning vectors designed to simplify commonly used cloning and sequencing procedures, including the construction of nested deletions for DNA sequencing, generation of RNA transcripts *in vitro* and site-specific mutagenesis and gene mapping. The pBluescript II phagemids have an extensive polylinker with 21 unique restriction enzyme recognition sites. Flanking the polylinker are T7 and T3 RNA polymerase promoters that can be used to synthesize RNA *in vitro*. The choice of promoter used to initiate transcription determines which strand of the insert cloned into the polylinker will be transcribed (Agilent Technologies, 2010).

The pBluescript SK/KS allow convenient blue-white screening of inserts (Short *et al.,* 1988). They posses versatile multiple cloning sites and can be used as sequencing and expression vectors (Figure 3). The pBluescript derivative pBloT7 contains an Nsi I

restriction site immediately adjacent to the T7 promoter sequence. Digestion of pBloT7 with Nsi I and subsequent removal of the 3' single stranded extension with pol Ik in the presence of all four nucleoside triphosphates creates a flush end immediately adjacent to the T7 promoter (Ramesh, 2000).

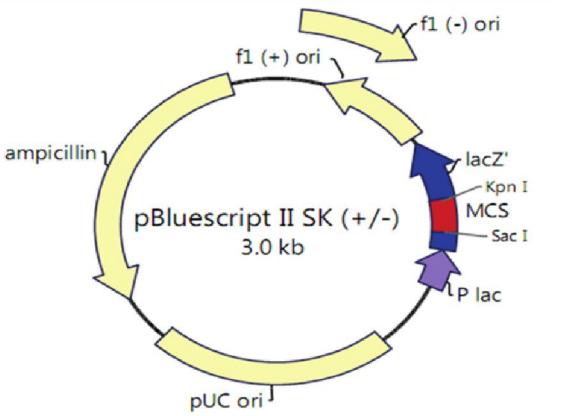

Figure 3: pBluescript II SK (+/–) multiple cloning site region.
Source: Agilent technologies (2010).

2.2. Recombination

Recombination is Exchange of genetic information between chromosomes or other molecules of DNA. In all cases of recombination, two DNA molecules are broken and rejoined to each other forming a crossover (Clark, 2005 and Bruce *et al*., 2002).

Foreign DNA is inserted into host cells by combining the foreign DNA with DNA of a vector. If the recombinant DNA gets inside a host cell, it can replicate along with the DNA of the host cell. This means that every time the host bacterium undergoes cell division, each new daughter cell receives a copy of the recombinant DNA, thus amplifying the recombinant DNA with each cell division (ACC, 2006).

In order to use a plasmid to insert foreign DNA into a bacterial cell, two steps are required: First, the foreign DNA must be combined with a plasmid. Secondly, a bacterial cell must absorb the recombinant plasmid. For the first step, restriction enzymes and DNA ligase are used. Restriction enzymes are naturally occurring enzymes that cut DNA. Many restriction enzymes are valuable tools in molecular biology. Each restriction enzyme cuts DNA only where a specific sequence of base pairs occurs. The broken bonds between the deoxyribose and phosphate groups that form the "siderails" of the DNA double helix (the phosphodiester linkages) must also be repaired. DNA ligase is the enzyme that catalyzes this reaction (ACC, 2006).

Recombination may be divided into homologous and non-homologous recombination. Homologous recombination is recombination between two lengths of DNA that are identical, or nearly so, in sequence where as non-homologous recombination is recombination between two lengths of DNA that are largely unrelated. It involves specific proteins that recognize particular sequences and form crossovers between them (Clark, 2005).

Site-directed mutagenesis also called site-specific mutagenesis or oligonucleotide-directed (Figure 4) mutagenesis is a molecular biology method that is used to make specific and intentional changes to the DNA sequence of a gene and any gene products. It is used for investigating the structure and biological activity of DNA, RNA, and protein molecules, and for protein engineering (Kilbey, 1995).

3. Overview Of Vaccine Development

The history of vaccination dates back to the 1798 studies by Edward Jenner, an English physician who used cowpox virus to immunize people against smallpox. Almost 200 years later, the comprehensive smallpox vaccination program established by the World Health Organization eventually led to the worldwide eradication of that disease. That success story is proof of the tremendous potential of vaccination and has led to the development of vaccines against almost all infectious agents affecting people and animals (Mark *et al*, 2008).

Veterinary vaccines are preparations containing antigenic substances which are administered for the purpose of inducing specific and active immunity against disease provoked by bacteria, viruses, or other microorganisms like parasites, funguses, antigenic fractions or substances produced by these organisms (DACA, 2006).

The development of rDNA procedures has provided some unique opportunities for vaccine production (OIE, 2008). This genetic modification can lead to the development of a new range of vaccines. These vaccines tend to be more stable, effective and safe (Sasidhara, 2006).

4. Classification of Recombinant DNA Vaccines

4.1. Plant based sub-unit vaccines

A novel approach for developing subunit vaccines has emerged as a result of the use of plants as host's biological bioreactors (Schuyler, 2008). Plant-based vaccines consist of protein subunits. A good candidate antigen must first be identified in order to develop the vaccine. Edible plant derived vaccines take advantage of the ability of some antigens to induce an immune response when delivered orally.

Foreign genes from disease agents are inserted into potatoes, soybeans, and corn plants and fed to animals and the expressed proteins from these foreign genes immunized the animals against the disease agent (Streatfield, 2005).

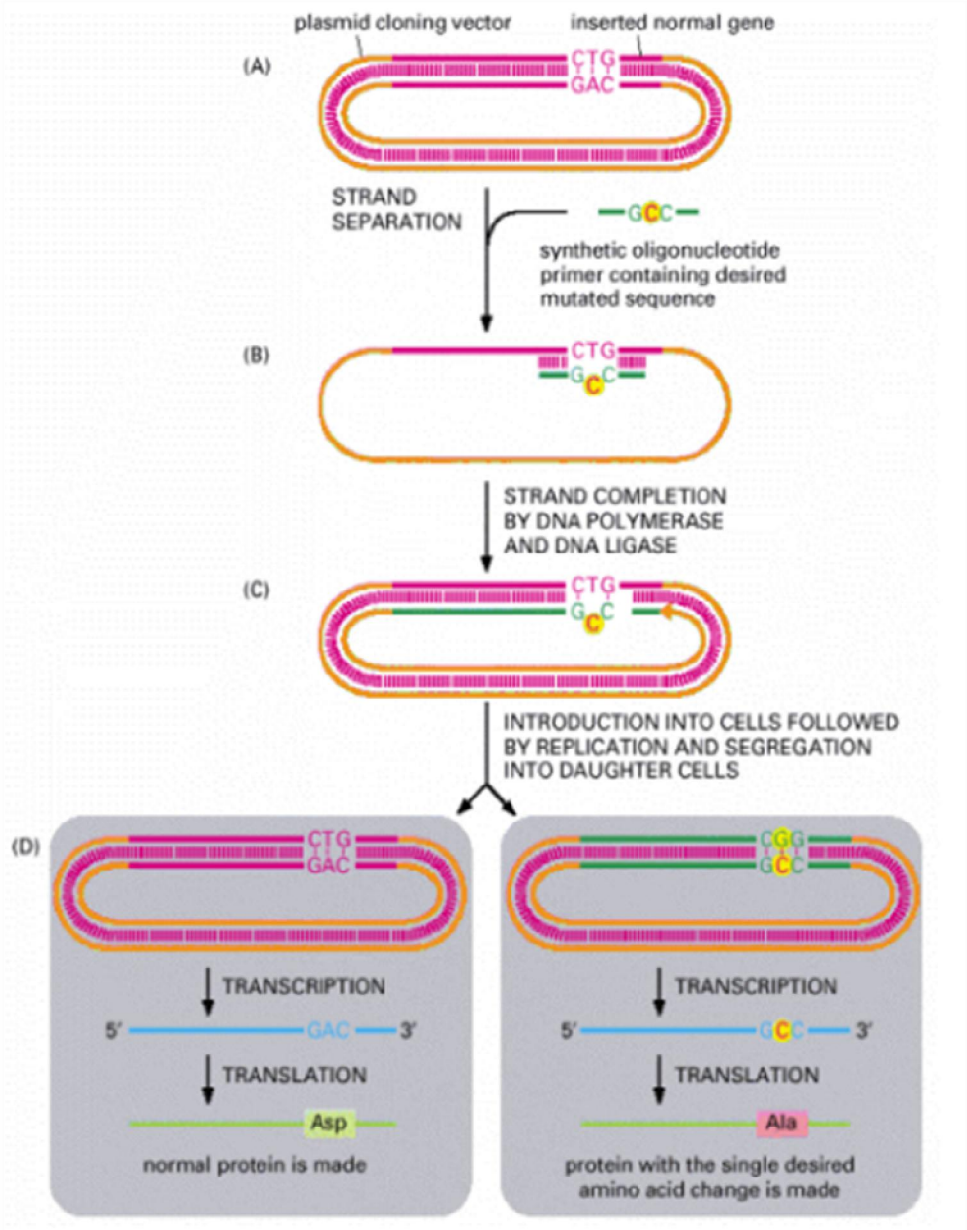

Figure 4: The use of a synthetic oligonucleotide to modify the protein- coding region of a gene by site-directed mutagenesis. **Source:** (Bruce *et al.,* 2002).

Edible plant-based vaccines eliminate the need for disposable injection materials and trained personnel to administer the injections. Moreover, farm animals do not need to be gathered to receive an injection; an edible product can conveniently be added to their regular feed. Certain types of plant material, such as grains store proteins in a dehydrated and stable condition for years at ambient temperatures, thus allowing for inexpensive storage and distribution. In addition, since plant-based vaccines are subunit vaccines, there tend to be fewer safety concerns than when considering live delivery vehicles (Streatfield, 2005).

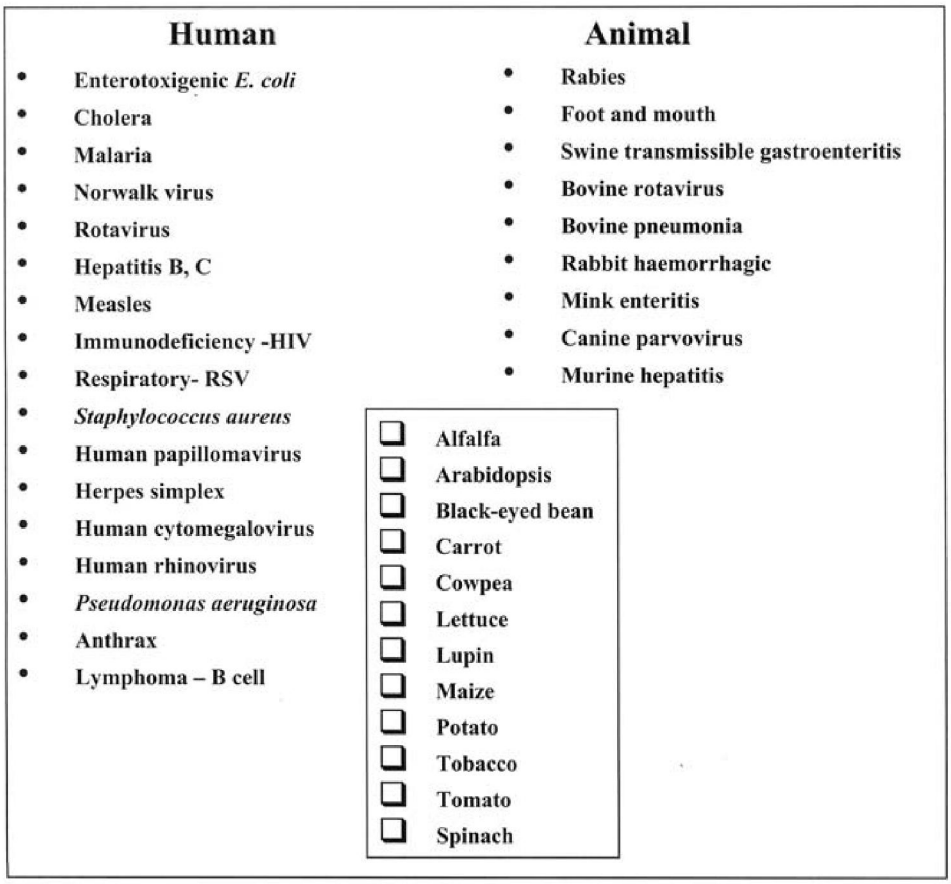

Figure 5: Subunit vaccine candidates against human and animal diseases for possible production in various crops. **Source**: (Schuyler, 2008).

4.2. Genetic vaccines

Genetic vaccines are circular pieces of DNA, called plasmids, which contain a foreign gene from a disease agent and a promoter that is used to initiate the expression of the protein from that gene in the target animal (Rodriguez and Whitton, 2000). The principle in designing any genetic vaccine consists in that a certain gene or region of the pathogen genome is incorporated into the carrier vector, which is subsequently used for vaccination. These vaccines provide the delivery of genetic material into the host cells and expression of the genes of the pathogen proteins in them. As a result, the pathogen antigens expressed by the cells in the organism are recognized by the immune system, which causes the induction of both the humoral and cell-mediated immune responses (Bessis *et al*., 2004).

In addition to genes coding for immunogenic proteins, genetic vaccines are designed to include different immune-stimulatory genes that trigger different compartments of the immune system, depending on the type of immunity desired. Unique features of DNA vaccines are intrinsic sequences embedded in the DNA, so-called CpG motifs. These unmethylated motifs were shown to act as an adjuvant, stimulating the innate immune responses and enhancing the effectiveness of the vaccine (Mark *et al*., 2008).

4.3. Recombinant inactivated vaccines

Recombinant inactivated vaccines are subunit vaccines containing only part of the whole organism. Subunit vaccines are synthetic peptides that represent the most basic portion of a protein that induces an immune response. Subunit vaccines consist of whole proteins extracted from the disease agent or expressed from cloned genes in the laboratory. Several systems can be used to express recombinant proteins, including expression systems that are cell free or that use whole cells. Whole-cell expression systems include prokaryotic (bacteria-based) systems such as *E. coli*, and eukaryotic (mammalian, avian, insect, or yeast-based) systems. Another type of recombinant subunit vaccines, called virus like particles (VLPs), can be created when one or more cloned genes that represent the structural proteins of a virus are expressed simultaneously and self assemble into VLPs. These VLPs are immunogenic. Because subunit vaccines do

not replicate in the host, they usually are administered with an adjuvant (Mark *et al*., 2008).

4.4. Live genetically modified vaccines

Live genetically modified vaccines could be viruses or bacteria with one or more genes deleted or inactivated, or they can be vaccines carrying a foreign gene from another disease agent, which are referred to as vaccine vectors. Deletion of a gene or genes is to inactivate or attenuate the disease agent. Generally two (double knockout) or more genes are deleted or inactivated so the vaccine remains stable and cannot revert to a pathogenic agent (Uzzau *et al*., 2005). Vector based vaccines are bacteria, viruses, or plants carrying a gene from another disease agent but can induce an immune response when the host is vaccinated with. For viral and bacterial vectors, the vaccine induces a protective response against itself (the vector) as well as the disease agent. Foreign genes must be inserted into the genome of the vaccinal vector in such a way that the vaccine remains viable (Mark *et al.,* 2008).

5. Recombinant Vaccines for Domestic Animals

Pathogens commonly vaccinated against in cattle are *Leptospira* sp., *E. coli, Clostridia* sp., *Mannheimia haemolytica* (also sheep and goats), *Haemophilus somnus*, infectious bovine rhinotracheitis, bovine viral diarrhea, bovine respiratory syncytial virus, para-influenza-3 (PI-3), rotavirus, and bovine corona virus. Vaccines used in sheep and goats include products for "overeating disease" caused by a bacterium, *Clostridium perfringens* types C and D, tetanus toxoid, sore mouth (contagious ecthyma) caused by poxvirus, diarrhea caused by *E. coli*, infectious causes of abortion in sheep (*Campylobacter fetus* and *Chlamydia psittaci*), footrot in sheep caused by two bacteria (*Bacteroides nodosus* and *Fusobacterium necrophorum*), and PI-3 respiratory disease in lambs (Mark *et al*., 2008).

The vaccine market for companion animals (Dogs, Cats and horses) is growing more rapidly than any other sector of animal health. This growth is caused partly by demographic changes in the human population and partly by the high value associated with companion animals. Canine vaccinations include those for *Leptospirosis*, kennel cough (*Bordetella bronchiseptica*), Lyme disease (*Borrelia burgdorferi*), rabies, distemper, adenovirus, parainfluenza virus, and parvovirus. Feline vaccinations target feline herpes virus, feline calcivirus, feline panleukopenia, rabies, feline leukemia, and feline immunodeficiency virus. Horses commonly are vaccinated for tetanus, rabies, equine influenza, and herpes virus infections. Recombinant vaccines are also being developed to decrease or eliminate economically important diseases in domestic swine and poultry (Dhama *et al*., 2008).

DNA vaccines against various animal diseases like foot and mouth disease and herpesvirus infection in cattle, Aujeszky's disease and classical swine fever in swine, rabies and canine distemper in canines, and avian influenza, infectious bronchitis, infectious bursal disease and coccidiosis in birds. For preventing viral diseases of the small ruminants, DNA vaccines have been developed that could protect against diseases like caprine arthritis-encephalitis, foot and mouth disease, Visna-Maedi and Rift valley fever (Dhama *et al*., 2008).

5.1. Commercially available recombinant vaccines

A number of recombinant vaccines are available commercially, and many more are projected to be available in the future (Mark *et al.,* 2008). Recombinant bovine vaccines have not been licensed for use in the United States. The European Union, however, has approved a naturally occurring glycoprotein E-deleted Infectious Bovine Rheinotrichiatis vaccine for its eradication program (Nobiron *et al.,* 2003). There are no commercially available recombinant vaccines used for sheep and goats in the United States. Currently, there are eleven United State licensed rDNA poultry vaccines (Table 1). Ten of these vaccines are live recombinant viral vectors designed to deliver specific genes to stimulate the host's immune system. Seven of these use an attenuated fowl pox virus as a vector to deliver selected pathogen genes. Three use an attenuated vaccine, Marek's disease virus, or a closely related nonpathogenic turkey herpesvirus as a vector. Surprisingly, only one license is for a bacterial pathogen, *Salmonella*, which is a double-knockout mutant resulting in a stable attenuated bacterium. Another interesting observation is that the list of pathogens being targeted by rDNA vaccines is small compared with the list of actual poultry pathogens (Mikalsen *et al.,* 2004).

Source: (Mark *et al*., 2008)

6. Limitations for Developing Recombinant DNA Vaccines

Limitations for developing recombinant DNA vaccines are shortage of capital, insufficiency of technology, and inadequate skilled and educated man power (Schuyler, 2008). Some of drawbacks of recombinant vaccines are: integrate into host genome, then by increasing the risk of malignancy (by activating oncogenes or inactivating tumor suppressor gene); induce responses against transfected cells, there by triggering the development of autoimmune disease; induce tolerance rather than immunity, and/or stimulate the production of cytokines that alter the hosts ability to respond other vaccines and resist infections (Hansson *et al.,* 2000; Hanlon and Argyle, 2001).

Table 1: Currently licensed rDNA poultry vaccines

Company	Pathogen	Vector
Lohmann AnimalHealth	Salmonella typhimurium	Double-deletion mutant
Merial Select	AIV, FPV	FPV
Merial Select	NDV, FPV	FPV
Merial Select	IBDV, MDV	HVT
Intervet	MDV	HVT
Intervet	MDV, NDV	MDV
Biomune Co.	AEV, FPV, LTV	FPV
Biomune Co.	AEV, FPV, LTV	FPV
Biomune Co.	FPV, MG	FPV
Biomune Co.	AEV, FPV, MG	FPV
Biomune Co.	FPV, LTV	FPV

AIV, avian influenza virus; NDV, Newcastle disease virus; IBDV, infectious bursal disease virus; MDV, Marek's disease virus; AEV, avian encephalomyelitis virus; FPV, fowl pox virus; LTV, laryngotracheitis virus; HVT, turkey herpes virus; MG, *Mycoplasma gallisepticum*.

Conclusion and Recommendations

Vectors are DNA molecules that are capable of replicating in the host cells and act as a carrier molecule for the construction of recombinant DNA; which covers yeast and bacterial artificial chromosomes, plasmids, phages, and blue script plasmids. Vaccines induce an immune response in the animal host that subsequently recognizes infectious agents and helps fight off the disease. Using recombinant DNA technologies, scientists have been able to develop plant based sub-unit vaccines, genetic vaccines, recombinant inactivated vaccines, and live genetically modified vaccines that no longer cause diseases yet induce a strong immune response. Developing vaccines using rDNA technologies requires a thorough understanding of the disease agent, particularly the antigens critical for inducing protection and the factors involved in causing disease. In addition, it is important to understand the immune response of the host to ensure that the vaccine induces the appropriate immunological reaction. There are recombinant vaccines for domestic animals; some of which are commercially available. However, the knowledge, immunization process and application of recombinant DNA vaccines are yet limited. The information's available regarding rDNA technology is less and the technology is not used in Ethiopia.

Based on the above conclusion, the following recommendations are forwarded;

- ❖ Further investigation and researches on recombinant DNA technology should be practiced.
- ❖ Since the technology is new, introduction to our country is highly recommended.
- ❖ The limitation of developing recombinant DNA vaccines should be studied thoroughly.
- ❖ Recombinant DNA vaccines are effective, cheap, thermo-stable and easily handled that meet the requirements of third world countries. Hence, the application and use of these vaccines must be practiced.
- ❖ All precautions and contraindications of veterinary vaccines should be clearly labelled by the manufacturers and every veterinarian should know and apply these carefully.

Acknowledgments

First and for most, we would like to praise God for all blessings in my life. Secondly, we would like also to thank my advisor Dr. Mebratu Asaye, for his intellectual advice, provision of necessary materials, and devotion of time for correcting and revising this manuscript. Thirdly, we need to extend my thanks to the coordinator Dr. Reta Tesfaye for his coordination of the course and necessary guidance. Last, but not least, my deep and special thanks also go to my families for their invaluable help and encouragement during my school/university life without any reservation. We finally express my thanks to my close friends who are at the side of me.

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