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Pros and Cons of Recombinant DNA Technology in Animal Diseases Diagnosis, Prevention and Control

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The advent of better diagnostics and vaccines are instrumentation in of animal health management programs prevention and control of many important diseases of livestock in a cost-effective manner. Most of the conventional vaccines have the limitations like residual virulence, extensive safety precautions, difficulty in production, cost constraints and sometimes requirement of specific growth conditions like cell associated nature of the pathogen. Further, increasing populations of both humans and animals, changes in demography and ecological disturbances have led to unprecedented succession of new pathogens which have the capacity to jump species barriers. However, recent advances in molecular biology, biotechnology, immunology, immunogenetics and genetic engineering have paved new ways in the development of more effective, safe and economical vaccines and diagnostics for not only to the conventional pathogens but also to the unprecedented virulent or exotic pathogens. In this review the authors have highlighted the recent developments in the recombinant DNA technology, its advantages and limitations and the future challenges in reference to the animal disease diagnosis and control.

Keywords: disease control, diagnostics, gene manipulation, economical, recombinant protein, vectors, vaccine.

Livestock have remained an integral component of the human society and development throughout the history. The sector, especially in developing and under-developed countries provides not only the supplementary source of income but also ensure high protein rich food source such as milk and meat to masses, and organic manure for crop production. Therefore, an organized planning and policy for livestock development will help in reducing poverty eradication, hunger and others objectives of the millennium development goals (MDG). However, increasing populations of both humans and animals and their relative densities, changes in demography, increase in transportations and relaxations in the trade by world trade organization (WTO), increased human to human and human to animal contacts which all led to increased chances for catastrophic epidemics like the recent swine and bird flu (Gretchen *et al*., 1996; Levin *et al*., 2007; AlHajjar and McIntosh, 2010). Also, we now face an unprecedented succession of new pathogens which have the capacity to jump species barriers to infect humans, and the associated frustration deriving from the inability to control such pathogens (Wack and Rappuoli, 2005; Riedel, 2006; Ma *et al*., 2008). There are many animal pathogens which are of zoonotic nature and have complicated epidemiology due to wild life reservoirs, carriers' hosts and arthropod vectors. The economically important and highly contagious disease of the animals include the foot and mouth disease (FMD), rabies, brucellosis, tuberculosis,

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haemorrhagic septicemia (HS), infectious bovine rhinotracheitis (IBR), bovine viral diarrhea (BVD), Peste des petits ruminants (PPR), Blue tongue, Brucellosis, Mastitis etc. and - infectious bursal disease, Marek's disease, infectious bronchitis (IB), egg drop syndrome, avian influenza of poultry which causes severe economic losses worldwide. For development of improved varieties of animals there have been use of biotechnologies in a wide range of production. Such technology has been used for the production of transgenic animals; profiling of DNA or better known as finger printings; animal tissue culture; diagnosis along with prevention and cure of diseases. Among the different biotechnological techniques the role recombinant DNA technique is unquestionable (Munro, 2001; Schroeder and Clare, 2003; Munshi and Sopari, 2004; Mepham, 2006).

Among the different measures, vaccination coupled with effective disease diagnosis remains the most important and cost-effective methods of disease prevention and control (McKeever and Rege, 1999; Babiuk, 2002). The eradication of smallpox in the 1970s globally and many others under consideration like polio, tetanus, diphtheria, measles, and hepatitis B, is only because of effectives vaccination programs (Ada, 2005; Nascimento and Leite, 2012). Such vaccines owe their success to their ability to target pathogens that have low antigenic variability and the protection depends mainly on antibody-mediated immunity (Nascimento and Leite, 2012). The main objective of livestock vaccines, on the other hand, is to improve overall production for the primary producers in a cost-benefit ratio (Babiuk, 2002). Currently, the majority of licensed bacterial and viral vaccines are either live attenuated or killed. However, conventional vaccines have the limitations of residual virulence, extensive safety precautions regarding personal and environmental contamination, difficulty in production, cost constraints, sometimes requirement of specific growth conditions and cell associated nature (Movahedi and Hampson, 2008). Advances in molecular biology, biotechnology, immunology, immunogenetics and genetic engineering have paved new dimensions to vaccinology and disease diagnosis, despite the threats posed by progressively virulent or exotic pathogens. All such effects have led to the health care and management in the livestock sector is all set to achieve a more significant position, intensifies by rapid diagnostic tools for epidemiological characterization of various pathogens and their interactions with the host along with the development of efficacious vaccines and immunostimulating agents to address the challenges posed by myriad pathogens.

The characteristics of the living organism is defined in context of the genome of all living beings that are made up of several genes. By the use of rDNA technologies it is possible to either modify or to delete the genes that are responsible to cause disease in an organism (Jackwood *et al*., 2008). Even though countless lives have been saved by vaccination it can have both favorable as well as unfavorable consequences. Certain types of vaccines especially the live vaccines have the tendency to revert back to pathogenic organisms thereby producing disease or in certain instances even death. New ways of attenuation of diseases has been provided by the development of rDNA technologies. This has been done by modification of the genetic makeup or genomes of the organisms for creating safer as well as more efficacious vaccines (Leong *et al*., 1995; Ellis, 1999; Rodriguez and Whitton, 2000).

However, it is to be kept in mind that at it is impossible to produce the ideal vaccine for any pathogen (Table 1). The authors herein briefly describe the recent developments in the recombinant DNA technology with reference to the animal disease diagnosis and vaccinology along with the limitations and future challenges.

Recombinant DNA technology

The recombinant DNA technology is the manipulation of the genome of the host bacteria, virus, yeast or plant to produce a desirable antigenic protein, glycoprotein or a peptide in the sufficient quantity to be used for the downstream applications like diagnostic, therapeutic or vaccination purposes (Mahon *et al*., 1998; Mustafa, 2001). The desired antigen is expressed in a secondary, preferably non-pathogenic, organism that is capable of expressing the immunogen in its native form or with minimal alteration so that it can be harvested using traditional antigen production methods, or delivered as a live non-pathogenic vector (Liljeqvist and Ståhl 1999; Rogan and Babiuk, 2005).

Due to advances in genomic and proteomic bioinformatics the rapid identification of protective epitopes has became possible including the cross-species identification of functionally similar proteins (Rogan and Babiuk, 2005). Recombinant DNA technology based products have many advantages over the conventional diagnostics and vaccines (Table 2). There is complete characterisation of the immunogen and the resulting product allowing commercial production and licensing regulations easy in a cost-effective and timely manner. The products eliminate the risks associated with handling of pathogenic organism and the risks associated with live or killed products like reverting to a pathogenic state (Babiuk *et al*., 2003; Belakova *et al*., 2007). It is also possible to generating fusion peptides with epitopes from mutiple pathogens to be used as single vaccine of inducing protective immunity for multiple pathogens (Whitton *et al*., 1993).

The various steps involved in the recombinant DNA technology for the production of desired antigen includes identification of gene of interest, its amplification by polymerase chain reaction (PCR), insertion in the desired vector and expression in the desirable expression host system (Fig. 1). Commercial production of a recombinant subunit vaccine requires the selection of an appropriate expression system based on the nature of the protein being expressed. There are various factors associated with the pathogen and host(s) which are to be considered while selecting the antigen to be used for the vaccination or diagnostic purpose (Table 3). Critical factors in selection of expression system include the production of an immunologically protective epitope, efficient and affordable downstream extraction and cleanup procedures, minimal immunological interference from host proteins, and minimal endotoxin contamination (Rogan and Babiuk, 2005). All these factors cannot be accumulated in a single system, however, depending on the requirements the following systems have been employed for the production of recombinant products for final use.

Bacterial expression

Prokaryotic expression is the most efficient and affordable for the production of a broad range of immunogens especially non-glycosylated proteins. Escherichia coli and Salmonella typhimurium are the most extensively used organisms for the expression of a wide variety of foreign due to the ease of handling and to their capacity for high level expression (Clark and Cassidy-Hanley, 2005; Wani, 2013). It can be tailored in such a way that the protein of interest is expressed on the surface of the bacteria, in the periplasm, as insoluble inclusion bodies or secreted in the media. However, there are many hindrences in the expression of glyco-proteins in which post-translational modifications are necessary (Babiuk, 1999; Clark and Cassidy-Hanley, 2005). Bacterial cells have codon preferences for which additional steps of codon optimization are required (Wani, 2013). The other hindrances include the presence of lipopolysaccharides the presence of which even in trace amounts has many complications including interference and possible injection-site reactions.

Yeast expression

The commonly used yeast for the expression of biologically active proteins used for vaccination and diagnostics of animal diseases is Saccharomyces cervisiae. There are many advantages of yeast expression compared to bacterial expression of the antigen. Yeasts are eukaryotic in natures and have the ability to express glycosylated protein production similarities to bacterial-based systems (Gerngross , 2004; Macauley-Patrick *et al*., 2005; Cregg *et al*., 2009). The can be cultured on simple media like bacteria and have lost cost of culturing, manufacturing production, scalability and ease of genetic modification. Also, the does not have cell wall therefore, pyrogen free (Gerngross, 2004; Rogan and Babiuk, 2005). The first ever subunit vaccine expressed, licensed and commercialized in the yeast expression system was for hepatitis B (Valenzuela *et al*., 1982; Adkins and Wagstaff, 1998).

Mammalian cell expression

The expression of some viral proteins especially the glycopreoteins can be carried out efficiently and economically only in mammalian cells. This is especially important for those glycoproteins where post-translational modification is important for proper folding and generation of specific epitopes (Zhu, 2012). Any type of cell line which is free from extraneous agents and preferably grows as suspension can be used for the expression. The expression of intracellular proteins or glycoptoeins in the mammalian expression system can be modified so that the final

product gets secreted out from the cell itself to make the downstream isolation and purification easy. This has further advantage to have less toxic effects on the cell itself and the transfected cells can be used for continuous expression of proteins.

Insect cell expression

Inserts act as biological vectors for many viruses and high virus titers can be achieved in their cell lines. The main aim of expression in insect culture is to have a high yield of immunologically active protein. Recombinant baculovirus are designed to express the gene product under the control of the strong polyhedron promoter, to produce glycosylated proteins except with added advantage to be accurately resembled like higher eukaryote glycosylation, than expressed in yeast expression system which is therefore believed to have a greater potential as protective immunogens (Rogan and Babiuk, 2005). However, there are many limitations of insect cell expression like inability to achieve high densities, higher requirements for dissolved oxygen and sensitivity to shear forces.

Plant-based expression

Plant molecular farming is recent approach where by genetic manipulations is carried out in plants to make them bioreactors for the production of various recombinant proteins (Obembe *et al*., 2011). Such plant based systems can represent ideal for the expression of protein in transient systems driven by well contained infectious vectors, or in stable transgenic systems based on nuclear or plastidial transformation (Buonaguro *et al*., 2010) with inducible or constitutive expression, seed-specific expression, plant virus-based and *Agrobacterium tumefaciens*-based transient expression systems (Rybicki, 2010). Plant based expression system eliminate the need of expensive fermentation, purification, cold storage, thereby reducing the overall manufacturing cost (Daniell *et al*., 2009). Plant-based expression in chloroplasts or the endoplasmic reticulum have the provisions for post-translational modifications viz. appropriate folding and disulfide bond formation (Moravec *et al*., 2007; Davoodi-Semiromi *et al*., 2009). One of the added advantage of expression of protein in the plants is part directed expression like in cereals, tubers, fruits or leaves which makes the further applications very economical and profitable. The stress inducing factor for the plant cells are the higher levels of expression of exogenous proteins. Several modern applications like selective promoters have been used for achieving higher levels of candidate antigens in the desirable parts of the plants. Tobacco is the most common plant used for protein expression because of its transforming ability. The ultimate goal to use transgenic plants as production systems for vaccine antigens in case of animals is to facilitate delivery of immunizing antigen in an easier way. This helps achieving immunization in mass against several diseases that are infectious in nature in a time bound manner (Rybicki, 2010; Obembe *et al*., 2011; Wani *et al*., 2011; Ahmad *et al*., 2012).

Advantages of recombinant DNA technology in veterinary vaccinology

DNA vaccines and cold chain requirement

The use of DNA vaccine was first proposed in 1990 in a report demonstrating that the purified bacterial plasmid DNA (Naked DNA) injected into muscles of animal host resulting in the expression of an encoded reporter gene (Moss, 2009). It has been envisaged that a simple vector less vaccine could be created by using a plasmid cassette containing an eukaryotic promotor and a gene or genes encoding the containing protective antigen (Weiner, 2008; Moss, 2009). They have the advantages of being easy to manufacture, less dose requirement per vaccination, don't require cold chain, completely safe and thus can be utilized for production of vaccines against deadly pathogens that are unsafe to handle and produced in large quantities (Dhama *et al*., 2008). DNA vaccines have the advantage of stimulating immunity both at humoral as well as cell mediated level and is not affected by presence of maternal antibodies in the young hosts (Fischer *et al*., 2003; Dhama *et al*. 2008). Targeted animal diseases for nucleic acid vaccines include FMD, IBR, BVD, TB, Brucellosis, Hog cholera, Rabies, Canine distemper, Brucellosis, Johne's disease; Avian influenza, IB, IBD, ND, MD and Coccidiosis (Dunham 2002; Dhama *et al*., 2008). The very first licensed DNA vaccine is against West Nile Virus which is a viral disease of horses. Other successful DNA vaccines are against Hematopoietic necrosis virus of salmon and canine melanoma (Dhama *et al*., 2008). One of the new approaches is self replicating vaccines which induces apoptosis of the cell and expose the expressed antigens transiently and are taken

by the antigen presenting cells for the development of cell media immunity (Ying *et al*., 1999; Jurgens *et al*., 2012). Molecular adjuvants like cytokines can be incorporated into such vaccines to improve their efficacies (Leong *et al*., 1994; Scheerlinck, 2001; Sasaki *et al*., 2003)

Subunit vaccines and undesirable toxic contaminants

Due to the short comings in the nucleic acid vaccination especially the limited expression of the exogenous protein in the host, it is desirable to express the protein outside first and inoculate the same for vaccination purpose. These protein antigens that are produced in a heterologous expression system like in bacteria or yeas cell and after purification are used as vaccine candidates (Jenkins, 2001; Clark and Cassidy-Hanley, 2005). Such exogenously expressed recombinant proteins have the advantage to be more immunogenic in nature. The vaccinated host produces antibodies to the protein antigen, thus protecting it from disease (Wani, 2013). Recombinant protein vaccines are based on the concept that humoral immune responses mounted to an infection are often targeted toward specific localized regions on the surface of protein antigens known as epitopes (Tizard, 2013). One of the major advantages of recombinant subunit vaccine is the product can be purified from the contaminants to a much higher degree and the production can be achieved at commercial level (Liljeqvist and Stahl, 1999; Jenkins, 2001; Clark and Cassidy-Hanley, 2005). In many laboratories most of the procedures of cloning have become routine as far as the development of sub unit vaccine is concerned. But the encoding DNA preparation proceeds with difficulty. It is therefore recommended that if natural DNA does not exist for a protein as for the proteins of many RNA viruses it is recommended to undertake enzymatic preparation of double-stranded complementary DNA by treatment of isolated RNA of the viral genome or messenger RNA with reverse transcriptase (Bachrach *et al*., 1983). Subunit vaccines have been developed for against avian influenza (AI), EDS, IBD, ND viral infection and avian coccidiosis in birds and Classical swine fever virus, parainfluenza Type 3, Mycobacterium tuberculosis, Bovine herpesvirus infection-1, foot and mouth disease, African horse sickness, rabies etc in animals. This is being used to try to develop new vaccines for difficult to vaccinate virus such as Ebola.

Mutant vaccines and environmental safety

Many biotechnological and molecular biological approaches are now being used to produce live culture vaccines with added advantages over conventional vaccines like complete deletion of the virulent genes and known mechanism of action and (Nascimento and Leite, 2012). Such biotechnologically engineered vaccines and mutant deletion vaccines are designed in such a way so that they can multiply under specific conditions either outside or inside the host only under certain conditions to make them unfit to grow under natural conditions (Frey, 2007; Carleton, 2010). They are made deficient in many biochemical processes or in the various proteins or dependence on substances like antibiotic genes. Mutant vaccines with such deficiencies make them highly deficient and dependent on the exogenous supplementation for growth. This helps in preventing the multiplication of pathogen after removal of the supplementation. Such mutant vaccines are being tried for diseases pasteurellosis, salmonellosis, listeriosis and others where cell mediated immune responses are more important and available vaccines are inducing only a short duration of immunity.

Recombinant vector vaccines and efficient cell mediated immunity

Although nucleic acid and subunit recombinant vaccines have many advantages over live attenuated vaccines they are still far off from inducing an efficient immune response as induced by live cultures. VectorVax of Zeon Corporation of Japan is the first commercial vector vaccine which is used in turkeys primarily. It contains a fowl pox vaccine virus carrying genes from Newcastle disease virus. Other agents that are used as foreign gene vectors are adenoviruses along with adeno-associated viruses (Streatfield, 2005). Recombinant vector vaccines are developed using large genome viruses like pox virus and lentivirus or by using bacterial vectors like attenuated strains of Salmonella and Mycobacterium bovis (Brochier *et al*., 1994; Tatsis *et al*., 2004; Bruhn *et al*., 2007; Bastos *et al*., 2009; Pincha *et al*., 2010). These are used to protect animals against various pathogens were cell mediated immune induction is necessary like in foot and mouth disease (FMD),

salmonellosis, avian influenza, MDV, NDV, IBDV etc (Roland *et al*., 2005; Rollier *et al*., 2011). Recombinant vector vaccines have the property that they can be used to deliver larger inserts of DNA (Leong *et al*., 1994; Draper and Heeney, 2010). Also, due to the properties like cellular tropism of Salmonella typhimurium for inductive sites of the immune system, cell-to-cell spreading and dissemination within the body manifested have many advantages. These help in the colonisation of specific mucosal surfaces or internal organs thereby stimulate both mucosal and systemic antibody production which is important in protection against causative agents that colonize mucosa or enter internal organs through the mucosa (Curtiss *et al*., 2010). There are also provisions that using S. typhimurium as vehicle for DNA vaccine to evaluate their possibility to develop oral vaccines for many pathogens (Cardenas *et al*., 1992; Curtiss *et al*., 2010).

DIVA / marker vaccines and disease monitoring

The evaluation of effectiveness of a disease eradication program can be achieved only by means of a method whereby carrier animal is differentiated from the vaccinated (immune) animals (Van Oirschot, 1999; Henderson, 2005). Also, current animal disease surveillance and eradication program are largely based on the serological tests for the confirmation of infection and the destruction of herds. These issues further limit the vaccination programs during out breaks in disease free zone and in endemic areas with potential of animal product trades (Babiuk, 2002; Ada, 2005) This can be achieved by developing vaccines that lack one or more antigens so that the antibody response produced by vaccinated animals is different from that of induced by a wild-type infection. Such, Differentiating Infected from Vaccinated individuals (DIVA) vaccines along with their companion diagnostic tests can play an important role in control of infections with simultaneous identification of naturally infected cases, ultimately leading to eradication of the pathogenic organisms (Pasick, 2004). DIVA vaccines have been tried for livestock diseases like Aujeszky's disease and others but there is still a long way to achieve the millennium development goals (Van Oirschot *et al*., 1986; Lee *et al*., 2011; Gao *et al*, 2012). In order to control an infectious disease the power to combine a marker vaccine along with a companion diagnostic kit depends on the performances of the vaccine and the kit themselves. It also depends on the way of using these tools by the authority who are competent in nature. By infection of the baby hamster kidney FMD vaccines are produced currently. The protection provided by these vaccines is derived mainly from antibodies that are induced against the virions which are inactivated. Such inactivated virions comprise of the structural proteins of the virus and the strength of this antibody response is an excellent indicator of the efficacy of the vaccine. There is also production of the non structural protein (NSP) of the virus during the process of cell culture replication and these can lead to antibody response if incorporated in sufficient amounts especially after booster vaccination. There is a strong antibody response to both viral structural proteins as well as NSPs because of infection in contrast to vaccination. Several techniques have been developed for removing most of the NSPs of FMDV during theprocess of production of vaccine. This use to make the vaccine marked negatively for these proteins so that antibodies against them can be regarded as indicator of infection but not vaccination (Doel, 2003; Clavijo *et al*., 2004; Uttenthal *et al*., 2010).

Reverse genetics and complex pathogens

One of the recent applications of genomics in vaccinology includes like identifying and cloning open reading frames (ORFs) that encode putative virulence factors and surface-localized proteins of a particular pathogen. This process is referred as reverse vaccinology and is used to predict several hundred ORFs of surface localization, their insertion in expression systems and comparisons to known vaccine candidates using a whole-cell enzyme-linked immunosorbent assay (ELISA) or fluorescent activated cell sorter (FACS) analysis (Sette and Rappuoli, 2010). Leading vaccine candidates are then tested in animal models or in vitro assays designed to provide some indication of the ability of the antigen to elicit a protective immune response. Another approach for viral disease prevention and control is by using virus like particles (VLPs) based vaccines (Lee *et al*., 2011). They are developed by expressing capsid proteins of viruses and then assembling them to form protein cages, which are

multivalent, similar to virus structures. These vaccines are infectious and resembling the actual viruses in their immunogenicity and are being tried for the animal diseases like avian influenza, ND etc (Webby *et al*., 2004; Hu *et al*., 2009; Lee *et al*., 2011).

Plant based oral vaccines and mass immunization

The use of transgenic plants as production systems for animal vaccine antigens has many advantages in animal immunization and vaccination programs. These vaccines have the potential to be cheap, safe, scale-up rapidly, easy to store and deliver, syringes and needle free, and eliminate trained personal for delivery (Tiwari *et al*., 2009). However, the most important and significant advantage is they can be produced in the edible portion of the localized plant thereby making the delivery easy to the masses in a short span of time (Shoji *et al*., 2011). They have the added advantage to induce both mucosal and systemic immune responses thus are very helpful in preventing the diseases of respiratory and gastrointestinal systems (Bae *et al*., 2003). The diseases against which such vaccines are under clinical and experimental trials include bovine rotavirus, parvovirus of canine, Brucella, *Bacillus anthracis*, transmissible gastroenteritis virus of swine, IBDV, NDV, avian reovirus , infectious bronchitis virus (IBV), avian influenza and others (Lamphear *et al*., 2004; Tiwari *et al*., 2009; Salyaev *et al*., 2010). These examples open the way for the development of an edible vaccine against other pathogen infection in livestock (Dhama *et al*., 2013).

DNA recombinant technology in animal disease diagnosis

One of the most important challenges in the disease control program is to respond promptly by diagnosing the disease causative agent. For the proper animal disease control program the diagnostic test should have several ideal properties. It must be easy to perform, requires no sophisticate instrumentation, allow decentralized implementation, no trained personal, no or minimal refrigeration requirements and to be economical and feasible for the farmers at the field level (Balamurugan *et al*., 2010; Wani, 2013). Traditionally diagnostic tools are being developed either to detect prevailing infections or to provide evidence of previous exposure to a given pathogen. The ultimate objective is to investigate and improve epidemiological information thereby increase disease awareness and make control and vaccination strategies easy. The latter continues to rely heavily on demonstrating specific antibodies, yielding information on sero-prevalence and advances in modern biotechnology especially by recombinant DNA technology have considerably enhance the efficiency of available detection systems (McKeever and Rege, 1999; Wani, 2013). The ideal antigens are probably the native proteins that provide epitopes which are sequence-specific as well as surface structural. Test antigens are essential for several diagnostic tests at present and are required to be produced continuously from cell culture or harvested from an animal which is infected. Such antigen preparations are expensive and often their shelf-life are short. Thus standardization is required for every new batch of antigen. Antigens produced by recombinant DNA technology have got several advantages over antigens that are produced from other biological sources. Such advantages include: high degree of purity along with high specific activity. Moreover since every preparation of the protein product is identical to the previous preparation batch-to-batch consistency is ensured (Ulrichs *et al*., 1998; Brune *et al*., 2000; Henderson, 2005; Hill *et al*., 2005). Most of such technologies are based on variations of enzyme-linked immunosorbent assay (ELISA) with its simplest form uses enzyme-conjugated secondary reagents. The applications of recombinant proteins in the ELISA and in the much simplified chromatographic techniques like lateral flow assays have paved new and economical ways. These techniques were being applied for the FMD, ND, rinderpest, peste des petits ruminants and many other viruses (Oem *et al*., 2009; Brüning-Richardson *et al*., 2011).

Limitations and challenges

The critical issue with regard to veterinary vaccines is costs per dose must be as low as possible to make the vaccination effective. Also, to combine all the factors of an ideal vaccine in certain type is very difficult. Factors like insertion of large segments of DNA in the nucleic acid vaccines and their expression systems are difficult. There is also chance of DNA integration in the host itself which may have many adverse consequences like malignancies, hereditary

complications and others. Constraints like expression of only non-glycosylated proteins, contamination by endotoxin pyrogens and other contaminations along with codon preferences to certain types of tRNA are associated with the bacterial expression (Wani, 2013). Low expression and hyperglycosylation of certain proteins and production of higher amounts of ethanol are limitations associated with the yeast (*S. cerevisiae*) expression systems. There are major question remaining with regard to plant expression system and the oral edible vaccines like how to move the vaccine through the anterior part of the gut (rumen and stomach) without degrading or inactivating the antigen itself. Other striking questions that require definite answer are evaluation of dosage requirements, possible plant cell interference with antigen presentation, immune tolerance, stability in stored fruit and other regulatory issues (Tiwari *et al*., 2009; Salyaev *et al*., 2010; Gilbert, 2009).

The various methodologies which are presently under intensive studies and research for efficient delivery of nucleic and recombinant protein acid based vaccines include recombinant vector based, virosomes based, chitosens based, virus-based nanoparticles (VNPs) and virus like particle (VLP) vaccination procedures. At the same time here is need to improve the immunogenicity of inactivated, recombinant, subunit or other types of vaccines by new variety of adjuvant strategies (Saade and Petrovsky , 2012). The expression of different innate immune system receptor ligands is one of such approaches to be used in third generation vaccines. The various molecules like TLR agonists (flagellin, bacterial derived CpG motifs, peptidoglycans etc.) and cytokines like IFNã, IL-2, IL-12 etc. are under active research. Approaches to mucosal vaccines include the incorporation of bacterial derived toxic moieties to the vaccine candidates like heat labile toxin or cholera toxin. These TLR agonists and other dander associated molecular pattern molecules (DAMP) are being tried with vaccines of NDV, CIAV and other subunit vaccines of poultry.

Conclusion and future perspectives

The ultimate aim of vaccines and diagnostics is to control the diseases in a cost effective manner. They must at the same time be profitable for manufacture and government agencies. Therefore, adequate partnership and collaboration between government, industry, health care organizations, and individuals in both academic and private sectors is necessary. For such long term goals recombinant DNA technology has to be optimally exploited for developing novel and highly efficacious vaccines; and for generating vaccines against new strains of existing pathogens and with multivalent vaccines for a single administration. Such vaccines based on genetic engineering techniques, may pave way for generation of much more efficacious vaccines that combat several diseases of animals in the coming decade. Although nucleic acid based vaccines and recombinant protein vaccines have many advantages there is an urgent need for the development of efficient delivery systems so as to stimulate the immune system fully. Efforts are needed to create disease free zones, by adapting mass vaccination programmes, keeping in mind a future eradication of major animal diseases. Research should target on high tech areas viz. genetic characterization of domestic animal species including establishment of their immunocompetent status and development of DNA markers for disease resistance traits and marker-assisted selection. All these targets require a multidimensional approaches for biotechnology and molecular biology along with immunology, immunogenetics and bioinformatics so as to achieve the Millennium Development Goals in a time bound fashion.

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