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REVIEW ARTICLE

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DNA VACCINES AND PREVENTION OF INFECTIOUS DISEASES IN BOVINES: A REVIEW

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ABSTRACT

Administration of plasmid DNA is considered a novel approach for developing effective immunoprophylactics for the control of infectious diseases in bovines. The major diseases of bovines for which the utility of DNA vaccines have been studied include brucellosis, tuberculosis, anthrax, foot and mouth disease, infectious bovine rhinotracheitis and bovine viral diarrhea. Research is in experimental stages for development of nucleic acid vaccines to counter many protozoan and rickettsial diseases also. The potential of DNA vaccines to overcome the maternal immunity in neonates, non-requirement of cold chain and their ability to generate vaccines that could differentiate infected from vaccinated animals, has heightened the prospects. Superior ability to induce cell mediated immune response has caught the attention of researchers involved in animal disease prevention. However, still there are many areas that need clarity and focused research for assessing the true immunogenic potential of DNA vaccines. Introduction of a variety of cytokines via recombinant DNA technology and the use of different immunomodulators and cationic lipids have shown good promise. Recently, efforts are being made to modulate antigen presenting cells so as to make the antigen presentation, a more efficacious one. The nucleic acid vaccines, having merits of being relatively stable, much safer and cost effective, are expected in near future to outplay the conventional vaccine methodologies that are commonly used to control bacterial and viral pathogens of bovines. In this review, the authors highlight the features of DNA vaccines, their utility and the prospects regarding use as an effective vaccination strategy for preventing infectious diseases of bovines.

Keywords: DNA vaccines, bovines, cattle, immunoprophylaxis, new generation vaccines, recombinant vaccines.

INTRODUCTION

The development of livestock, especially in developing and under-developed countries would not only provide supplementary source of income but also ensure high protein rich food source such as milk and meat for masses, and organic manure for crop production. Animal health care is a key component for successful animal production and the main limitation to an effective livestock health management is the inadequate focus on preventive measures. For prevention of foot and mouth disease, infectious bovine rhinotracheitis, tuberculosis, anthrax, brucellosis and many other important diseases of bovines, immunization with vaccines has been practiced, which usually comprises of killed/inactivated or attenuated/live pathogens. Still, a complete prevention or eradication of many infectious agents has not been accomplished. Livestock disease control has undergone a paradigm shift in recent years and animal science research over the last few decades has paved way for development of technologies in the areas of animal health care and management to evolve new generation preventive measures. DNA vaccines or nucleic acid based vaccines, one among the third generation vaccine approaches, possess several distinct advantages, which include ease of manufacture, possibility of manipulation using genetic engineering techniques, superior stability, much safer and cost effective and the ability to work in presence of maternal antibodies, when compared to the conventional vaccines. For killed or inactivated vaccines, the cellular immune responses are poor and in live attenuated ones both the cell mediated immunity (CMI) and humoral immune responses (HIR) are elicited. But the live vaccines have demerits like reversal of virulence of pathogen, cumbersome production procedures, handling of

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hazardous organisms etc. which can be easily solved by nucleic acid vaccines to a great extent. Apart from this, several different genes can be combined, making it possible to vaccinate against several pathogens at the same time. The demerits of DNA vaccines, at theoretical levels and yet not scientifically proved, are integration into host genome and causing activation of proto-oncogenes or inactivation of tumor suppressor genes and also the generation of anti-DNA antibodies resulting in autoimmune diseases (Hasan *et al*., 1999; Sharma and Khuller, 2001; Dunham, 2002).

The concept of DNA vaccine was first evolved by Wolff *et al*. (1990), when it was found that intramuscular (IM) injection of a recombinant bacterial plasmid DNA yielded the expression of a gene in mice. This lead to the development of nucleic acid based vaccination technique, which is currently an effective way for the generation of protein *in vivo* for the initiation of immune response (Tang *et al*., 1992; Ulmer *et al*., 1993; Dufour, 2001; Reyes-Sandoval and Ertl, 2001; Liu, 2003; Rogan and Babiuk, 2005; Ulmer *et al*., 2006). The desired immune responses viz. CMI and HIR are generated with these vaccines (Liu, 2003; Liu *et al*., 2006). It is also an attractive option due to the capability of easier bulk production in bacteria and manipulation using recombinant DNA techniques (Wolff and Budker, 2005). As an effective prophylactic strategy, plasmid DNA contains gene encoding the antigenic determinant of a specific pathogen which when injected into a host, is translated and transcribed into a peptide that elicit protective responses, nearly mimicking a live infection in the host. While immune responses to DNA vaccines alone, in some instances, have been found relatively weak compared to that elicited by conventional live vaccines. But the combinations with adjuvants or with recombinant proteins in prime-boost (DNA vaccine priming and recombinant protein boosting) regimen have got appreciable protective responses (Ramshaw and Ramsay, 2000; Radcliffe, *et al*., 2006). As the manifold merits of nucleic acid vaccines unfold, recent data suggest that they have moved towards second stage clinical trials for preventing many diseases affecting humans. DNA vaccine technology has shown promising results in the treatment of diseases like acquired immunodeficiency syndrome (AIDS), herpes infections, hepatitis B and C, influenza, rabies, rotaviral infections, Ebola, tuberculosis, malaria, Leishmaniosis, mammary and lung carcinomas and autoimmune diseases like multiple sclerosis and rheumatoid arthritis (Robinson *et al*., 1993; Lu *et al*., 1995; Xiang *et al*., 1995; Lowrie *et al*., 1997; Wang *et al*., 1998; Fonseca *et al*., 2001; Bulut *et al*., 2003; Reisfield *et al*., 2004; Quaglino *et al*., 2004; Karin, 2004; Vastag, 2004). In contrast, the research for the application of DNA vaccines in veterinary pracitce has been on a lesser pace. But during the last few years many trials for the development of DNA vaccines have been conducted for developing effective immunizing agents against animal pathogens (Lewis *et al*., 1997; Babiuk *et al*., 1998; Lewis and Babiuk, 1999; Dunham, 2002; Ravindra *et al*., 2004; Rogan and Babiuk, 2005; Liang *et al*., 2006; Babiuk *et al*., 2007). Targeted diseases are foot and mouth disease (FMD), infectious bovine rhinotracheitis (IBR), bovine viral diarrhea (BVD), tuberculosis, brucellosis etc. in cattle; Aujeszky's disease and hog cholera in swine; rabies and canine distemper in canines; caseous lymphadenitis, brucellosis, Johne's disease, caprine arthritis, encephalitis in small ruminants, and avian influenza, infectious bronchitis, infectious bursal disease and coccidiosis in avian species (Chaplin *et al*., 1999; Lillehoj *et al*., 2000; Kodihalli *et al*., 2000; Oshop *et al*., 2002; Cheevers *et al*., 2003; Vannier and Martingant, 2005; Ding *et al*., 2005; Yang *et al*., 2005; Gupta *et al*., 2006; Sechi *et al*., 2006; Patial *et al*., 2007). One of the distinct advantage of the DNA vaccines in the veterinary field is the possibility of utilizing the concept of differentiating infected from the vaccinated ones (DIVA), which makes it suitable to effectively eradicate a pathogen from the environment. This utility has been exploited in diseases like FMD and IBR (Toussaint *et al*., 2005; Grubman, 2005) and should be extended towards BVD and brucellosis, in which the international trade regulations are strictly followed. The potential of DNA vaccines to overcome the maternal immunity is also a remarkable feature, which could be attributed to that fact that the DNA immunized might be refractory to the maternal antibodies, and the dendritic cells have the ability to provide a durable source of the antigen (Manickan *et al*., 1997).

Keeping in view of the advances, utilities and ease of generation of the DNA vaccines, the present review highlights the salient features and advancements in DNA vaccinology, and its applications in preventing infectious diseases affecting bovines.

SALIENT FEATURES OF DNA VACCINES

DNA vaccines, when compared to peptide or recombinant protein based subunit vaccines, are cost effective, stable and easily transportable in lyophilized form which is having more significance in tropical countries. They comprise of circular bacterial plasmids with desired antigen gene under the transcriptional control of viral or eukaryotic promoter along with a poly-adenylation (poly-A) signal sequence and bacterial origin of replication (Davis, 1997; Gurunathan *et al*., 2000) (Fig. 1). Promoter sequences most commonly used are of viral origin especially of Cytomegalovirus (CMV), Rous Sarcoma Virus (RSV) and Simian Virus 40 (SV40), among which

CMV promoter is the preferred one (Galvin *et al*., 2000; Sharma and Khuller, 2001). For selection in bacteria during the production process, the plasmid contain an antibiotic resistance gene (Gurunathan *et al*., 2000; Liu, 2003; Brandsma, 2006) and for the optimization, the presence of Kozak sequence (GCCA/GCC), and an SV 40 enhancer region are desirable (Kozak, 1997; Xu *et al*., 2001). Chimeric plasmids are capable of *in vivo* expression and presentation of desired viral, bacterial or parasitic antigens, and to induce specific immune responses.

Gene gun based administration of DNA vaccines requires lesser amounts of plasmid DNA as compared to needle based intramuscular (IM) immunization (Yang *et al*., 1990; Hasan *et al*., 1999; Perrin *et al*., 1999; Dunham, 2002). Alternative delivery strategies include suppositories (Loehr *et al*., 2001), needle free injector system (van Rooij *et al*., 1998), electroporation (Mir *et al*., 1999), mucosal delivery (Barnes *et al*., 2000) and topical application (Oshop *et al*., 2002). During application using 'gene gun', *via* IM route, the recombinant plasmid DNA coated onto gold microbeads gets directly transfected to dendritic cells and epidermal keratinocytes, which leads to an effective antigen presentation (Porgador *et al*., 1998; Gurunathan *et al*., 2000; Dunham, 2002). On the contrary, the needle based IM inoculation often results in predominant transfection of myocytes while the transfection of dendritic cells is relatively lesser (Kovacsovics *et al*., 1993; Manam *et al*., 2000; Donnelly *et al*., 2005). Electroporation enhances the activity of antigen presenting cells (Babiuk *et al*., 2004) and delivery via the mucosal route helps in the development of mucosal immunity which is critical in providing a barrier against pathogens that enter host via mucosal route. Topical DNA vaccination also has been shown to elicit both humoral and cellular immune responses *in vivo*, but require delivery systems to improve permeability of epidermis (Oshop *et al*., 2002). To enhance the effects of DNA vaccines, improvements have to be made in various delivery formulations or develop newer formulations so that generation of these vaccines against infectious pathogens could materialize. DNA vaccines activate both the arms of the immune system and have been found effective in a variety of animal model experiments (Montgomery *et al*., 1994; Iwasaki *et al*., 1997; Hasan *et al*., 1999; Sharma and Khuller, 2001; Dunham, 2002; Babiuk *et al*., 2003; Babiuk *et al*., 2007). After the *in vivo* generation, the antigenic peptides are processed and presented to the cells of the host immune system. Professional antigen presenting cells (APCs), especially dendritic cells play a pivotal role in generation of immune response to DNA vaccine. They are primed either by direct transfection with plasmid DNA or indirectly by the transfected myocytes via cross presentation (Fu *et al*., 1997; Hasan *et al*., 1999; Corr *et al*., 1999; Dunham, 2002; Stevenson, 2004). In myocytes or dendritic cells, the synthesized protein allows the presentation by MHC I, while the uptake of proteins by APCs allows presentation via MHC II, thus inducing both cellular and humoral immunity (Sharma and Khuller, 2001).

Still a vivid picture is not there regarding the mechanism of cellular transfection and the development of immune responses. The major lacuna that impedes the flourishing of this new generation vaccine is the inability at times to generate desirable levels of immune responses. So the quest for higher immune responses led to experimenting different approaches for formulating DNA vaccines, meant to protect the DNA from degradation and improve the transfection efficiency via various formulations using cationic lipid complexes and adjuvants (Manoj *et al*., 2004a; Jang and Shea, 2006; Rao *et al*., 2007; Greenland and Letvin, 2007). For mucosal or oral immunization, the best formulations for the delivery of DNA vaccine are liposomes (Sharma and Khuller, 2001; Liu, 2003). Also, cytokines (IL-2, IL12, IL-15, IL-18, IFN-β, GM-CSF) have proven useful new generation adjuvants (Haddad *et al*., 2000; Sharma and Khuller, 2001; Liu, 2003; Lillehoj *et al*., 2005).

For generating sufficient immune responses with DNA vaccines, much depends on the delivery system that point towards a higher transfection efficiency in *in vivo* conditions. To address this situation, various options to improvise DNA vaccines have been analyzed for significantly increasing the levels of neutralizing antibodies while preserving the cellular immune responses (Fu *et al*., 1997; Chaplin *et al*., 1999; Rice *et al*., 1999; You *et al*., 2001; Babiuk *et al*., 2003; Manoj *et al*., 2004a; Hauser *et al*., 2004; Talsma *et al*., 2006). The modification of the properties of dendritic cells and increasing their functional life is a novel strategy that could also be helpful for enhancing the potency of DNA vaccines (Tsen *et al*., 2007). Immunity enhancing properties have also been noticed for the plasmid vector containing unmethylated CpG sequences (CpG motifs) (Klinman *et al*., 1997; Krieg *et al*., 1998; Sharma and Khuller, 2001; Dunham, 2002; Krieg, 2002).

NUCLEIC ACID VACCINES IN BOVINES

The last decade have seen the development of recombinant plasmid based DNA vaccines, using immunogenic genes of pathogens, against many diseases like infectious bovine rhinotracheitis, bovine viral diarrhea, foot and mouth disease, tuberculosis and brucellosis (Fig. 2). These third generation vaccines constitute a revolution in the concept of immunoprophylaxis, which was previously based on the inoculation of a live attenuated or

inactivated organism. It is now possible to induce immunization by direct injection of the gene that codes for the immunogenic antigen. Among many advantages, DNA vaccines also provide differentiation of infected from vaccinated animals (DIVA) and hence vaccine-induced herd immunity can be measured during animal vaccination programmes (van Oirschot, 2001; Pastoret and Jones, 2004). It also generates suitable vaccines that over comes maternal immunity which can be used to immunize young ones of domestic animals at a very early age. Keeping in view the merits of DNA vaccines, the latest research developments in DNA vaccine technology for preventing the infectious diseases in bovines is presented herewith.

Genetic immunization approach against bacterial diseases of bovines offers attractive possibilities for rapid and effective vaccine development. Researchers have successfully developed nucleic acid vaccine based immunoprophylactic strategies to control microbial infections like anthrax (Galloway and Baillie, 2004). Anthrax is a well-known zoonotic disease and was one of the first to be described in association with its causative organism, *Bacillus anthracis*, the principal virulence factor of it being a multicomponent toxin consisting of three separate gene products designated protective antigen (PA), lethal factor (LF), and edema factor (EF). Anthrax is most common in domestic herbivores but also affect humans exposed to tissue from infected animals, contaminated animal products or directly to bacterial spores (Friedlander *et al*., 2004; Galloway and Baillie, 2004). The disease in cattle is characterized by abrupt and high fever, a period of excitement followed by depression, respiratory or cardiac distress, staggering, convulsions, and at times, the course of disease is so rapid that illness is not observed and animals are found dead with bloody discharge from natural orifices. Protection against anthrax infection is associated with humoral immune response directed against protective antigen and also it is suggested that lethal factor, and edema factor may also contribute to specific immunity (Gu *et al*., 1999; Price *et al*., 2001). For the prevention of this disease in herds, live non-capsulated Sterne's vaccine is used almost universally (Friedlander *et al*., 2002). Recently, there is substantial interest in the development of a more highly defined DNA-based anthrax vaccine for genetic immunization, due to the potential advantages associated with this approach, and numerous efforts directed towards achieving the goal are in progress. Ivins *et al*. (1995) has reported the usefulness of DNA vaccines in combination with adjuvants against anthrax during his challenge studies in guinea pigs vaccinated with PA gene. Later, it was also demonstrated that one can obtain protective response to a spore challenge by immunization with a plasmid encoding the 63-kDa protease-cleaved fragment of PA (Price *et al*., 2001). Hahn *et al*. (2004) reported the development of protective antigen based DNA vaccine that could protect the experimental animals against *B. anthracis* challenge. Monovalent and bivalent anthrax plasmid DNA (pDNA) vaccines encoding genetically detoxified protective antigen (PA) and lethal factor (LF) proteins have also been successfully tested for their immunogenicity and ability to protect rabbits from an aerosolized inhalation spore challenge (Hermanson *et al*., 2004).

Brucellosis, known as contagious abortion or Bang's disease, is a zoonotic disease that causes abortions in cattle, the transmission of the disease occurring by ingestion of organisms from contaminated sources, or by entry through mucous membranes or conjunctivae (Kurar and Splitter, 1997; Schurig *et al*., 2002). Although the rate of occurrence is decreasing due to vaccination of animals, the disease has not been still eradicated from many countries. Particularly in developing and under developed countries, where domestic ruminants are essential to the economy, brucellosis is a major cause of direct economical losses and a major impediment for trade and exportations. Brucellosis is usually controlled by mass vaccination of livestock with strain 19, RB 51 and 45/20 vaccines which have been found to increase the resistance of herd to the infection, but individually have many demerits (Stevens *et al*., 1994; Schurig *et al*., 2002). To overcome the drawback, nowadays, nucleic acid based vaccines that could modify the protective antigens are being tried, which could solve this problem to a great extent. Against brucellosis, an IM administered L7/L12 gene has been reported to result in intracellular expression of the immunodominant L7/L12 protein, which for the first time focused attention on DNA immunization against this disease in bovines (Kurar and Splitter, 1997). In another study, Rosinha *et al*. (2002) suggested that *Brucella abortus* glyceraldehyde-3-phosphate-dehydrogenase (GDPH), a T and B cell reactive protein, could induce partial protection when co-administered with IL-12. The Cu-Zn super oxide dismutase (SOD) antigen of *B. abortus*, a T cell antigen has also been utilized for the generation of an effective and suitable DNA vaccine (Gonzalez *et al*., 2006; Rivers *et al*., 2006). Aside to all these works related to developing effective and safer vaccines, to ensure DIVA and thereby an effective eradication of the disease is of paramount importance. For this, development of DNA vaccines with specifically modified immunogenic genes should be the prime focus of the research community.

Bovine tuberculosis, caused by *Mycobacterium bovis*, is a major economic problem around the world and

poses a significant threat to public health even though pasteurization has succeeded in minimizing bovine tuberculosis (TB) transmission to human beings. Currently, the only control measure for this chronic disease in animals is a 'test-and-slaughter' policy based on tuberculin sensitivity testing. In bovines, TB is manifested as progressive emaciation, lethargy, weakness, anorexia, and mild fever (Huygen, 2003). The bronchopneumonia associated with the disease causes a chronic, intermittent, moist cough with later signs of dyspnea and tachypnea. For the control of bovine TB, Bacillus Calmette-Guerin (BCG), an attenuated strain of *M. bovis* is presently the only available vaccine. However, the reported protective efficacies of BCG vaccination in cattle have differed greatly, ranging from meager levels to about 70% protection. Cell-mediated immunity (CMI) is essential for the control of mycobacterial infections and due to the strong cellular imminity they can induce, DNA vaccines are being considered for use against mycobacterial infections. Huygen (2003) has clearly explained the utility and feasibility of DNA vaccines in providing significant immune response against this pathogen. Based on *Myobacterium bovis* protein MPB-83, a DNA vaccine tested in mice was found to elicit protective immune responses (Chambers *et al*., 2000). The utility of the conjugation of co-stimulatory molecule CD 154 along with protective antigens has also been reported to enhance the immunogenic properties of mycobacterial DNA vaccines (Maue *et al*., 2004). *M. bovis* Ag85B gene was also found suitable while incorporating in plasmid vector due to its ability to induce a Th1 type of immune response (Teixeira *et al*., 2006). Aside to this, many workers have shown that DNA vaccines used in combination with BCG give superior protection against experimental *M. bovis* challenge in cattle (Vordermeir and Hewinson, 2006; Cai *et al*., 2006).

One of the most economically important diseases that affect the dairy industry is bovine mastitis. Even though multiple etiologies have been recorded, *Staphylococcus aureus* is considered to play a significant role in the infection. *S. aureus* is a contagious pathogen that often results in chronic intra-mammary infections in dairy cows and has the ability to invade the mammary gland by employing many virulence factors, including α-toxin, β-toxin and Protein A (Shkreta *et al*., 2004; Kerro-Digo *et al*., 2006). As a preventive strategy, vaccines are being used but the current vaccine formulations have been found ineffective in preventing the infection primarily due to the antigenic diversity of the bacterium. So, many workers have attempted to stimulate an immune response in dairy cows through injection of plasmid DNA designed to express staphylococcal proteins. For the control of staphylococcal mastitis in bovines, the fibronectin binding protein (FnBP) and clumping factor A (ClfA) involved in the pathogenesis of *S. aureus* are considered important proteins to look upon for developing DNA vaccines. A DNA vaccine comprising of a bicistronic plasmid encoding the fusion of FnBP and ClfA genes has been reported to induce sufficient protection against *S. aureus* infection (Shkreta *et al*., 2004). Another nucleic acid vaccine with ClfA alone was also found to induce a strong and specific antibody response (Nour El-Din *et al*., 2006). Kerro-Dego *et al*. (2006) has developed DNA vaccines based on Gap B and Gap C protein genes of *S. aureus* and showed that utilizing a prime boost strategy involving the proteins could elicit significant protection against the pathogen.

Against viral diseases of bovine, especially infectious bovine rhinotracheitis (IBR), bovine viral diarrhea (BVD) and foot and mouth disease (FMD), researchers have tried many DNA vaccine approaches. IBR is an economically important disease of bovines caused by bovine herpesvirus-1 (BHV-1), an *Alphaherpesvirus* responsible for clinical infection and restrictions in international trade. A variety of clinical syndromes in cattle, including respiratory, genital, nervous and multisystemic infections have been reported due to BHV infections (Edwards *et al*., 1983; Edwards *et al*., 1990). Infection of the upper respiratory tract is associated with fever and drop of production and also facilitates secondary infection by bacterial pathogens, leading to bronchitis or pneumonia that could be fatal (Toussaint *et al*., 2005; Zheng *et al*., 2005; Huang *et al*., 2006). Infectious balanoposthitis and infectious pustular vulvovaginitis, affecting the reproductive status of the animals are also associated with the infection. Vaccination with conventional live attenuated or inactivated vaccines has been the predominant control strategy against BHV-1 (Bosch *et al*., 1998; Mars *et al*., 2001). But, the currently used inactivated and modified live BHV-1 vaccines do not confer adequate protection against infection, and found ineffective in stopping the spread of the virus (Zatechka *et al*., 1999). Moreover, the ability of the virus to down regulate the expression of MHC class I molecules (Nataraj *et al*., 1997) and to induce apoptosis of $CD4^+$ T lymphocytes (Eskra and Splitter, 1997) results in defective CMI responses. This scenario warrants the need to develop methods to induce effective CMI responses to clear the virus from the host. DNA vaccines are associated with many advantages that render them very attractive for performing T cell priming and to be used for developing prime-boost strategies that could facilitate and additional advantage to vaccination against IBR (Toussaint *et al*., 2005). Many workers have extensively studied and developed successful DNA vaccine strategies against BHV-1 infection (Gupta *et al*., 2001;

Table 1: Advantages of DNA vaccines compared to conventional vaccines

1	Vaccine can be developed against hazardous pathogens
1	A single plasmid can encode multiple pathogen genes
1	Cytokine adjuvants can be co-administered
1	Variety of immunomodulators can be used
1	Bacterial plasmids have inherent immunogenic properties
1	Can be used in prime-boost regimen
1	DIVA strategy can be utilized for disease eradication
1	Provide long-lived immune response
1	Elicits cell mediated and humoral immune response
1	Able to overcome effects of maternal antibodies
1	Multiple administration is not needed
1	Normal saline or PBS can be used as vehicle
1	Much easier production process in bacteria
1	Safer to handle in laboratory conditions
1	Cost effective
1	Extremely stable

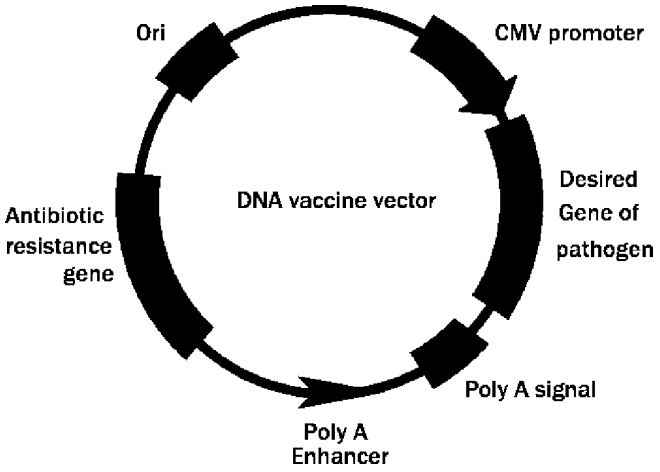

Fig. 1: Pictorial illustration portraying the essential elements for a suitable DNA vaccine vector.

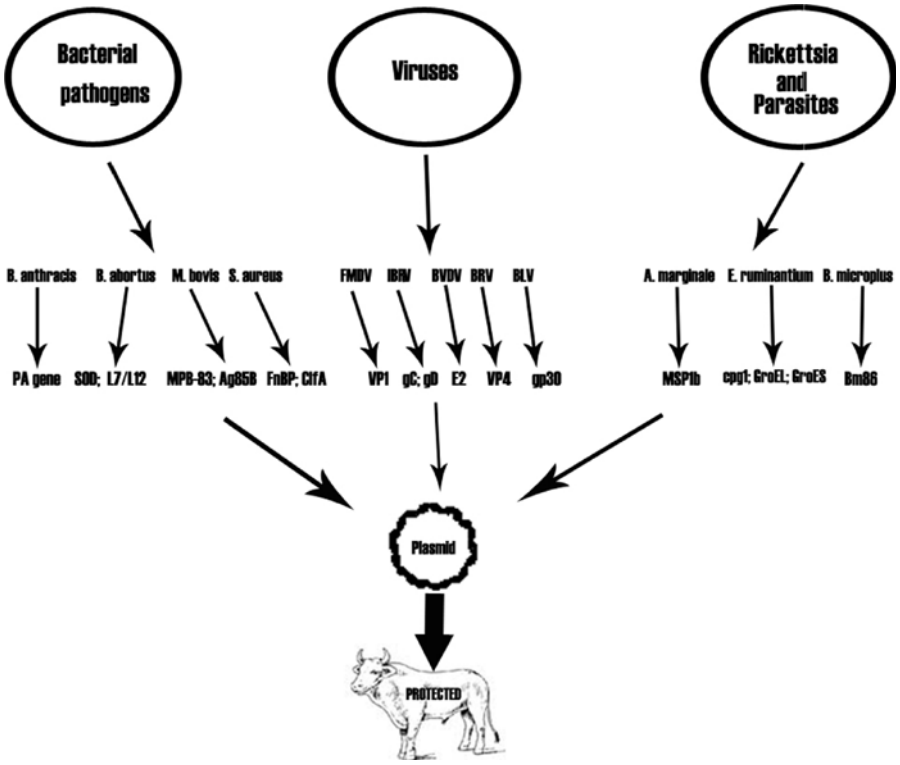

Fig. 2: Diagrammatic representation of the genes of bovine pathogens used in DNA vaccine formulations.

Castrucci *et al*., 2005). Gupta *et al*. (2001) reported the DNA immunization with gC gene could induce neutralizing antibody and lymphoproliferative responses in bovines. Also, BHV-1 glycoprotein B along with IL-12 has been found to enhance the CTL responses (Huang *et al*., 2006). For the induction of mucosal immunity, the use of suppositories containing plasmid coding for glycoprotein D can also been tried (Loehr *et al*., 2001). The potential use of CpG-enhanced plasmid was exploited for developing BHV-1 glycoprotein D based DNA vaccine which was found to induce desirable immune responses in cattle (Pontarollo *et al*., 2002). Bovine CD 154 linked to glycoprotein D can induce enhanced immune responses in cattle against herpes virus infection (Manoj *et al*.,

2004b). Recently, it has been suggested that gD of BHV-1 confers higher protection when compared to gC glycoprotein (Toussaint *et al*., 2005). In another study, the intercellular trafficking ability of BHV-1 VP22 protein has been exploited to improve the efficacy of a DNA vaccine encoding glycoprotein D (Zheng *et al*., 2005).

Bovine viral diarrhea (BVD) is another important disease of bovine which has initiated national and regional control and eradication campaigns against the BVD virus, which belongs to genus *Pestivirus* of family *Flaviviridae*. BVD is most common in young cattle and generally accompanied by typical mucosal lesions and diarrhea (Baker, 1995; Nobiron *et al*., 2003). Test and removal strategy by strictly following fundamental elements of biosecurity, removal of infected animals and monitoring of herd status and systematic vaccination are the acceptable method for eradication of this virus. The E2 protein of BVDV plays a major protective role against BVDV infection (Harpin *et al*., 1999). Recently, efforts are targeted towards developing new generation vaccines against this economically important disease. DNA vaccine that could protect the bovine population against BVD has been developed. Plasmid DNA expressing the BVDV type 1 major glycoprotein E2 has been found to induce virus-specific neutralizing antibodies (Harpin *et al*., 1999; Nobiron *et al*., 2003). Non-structural protein NS3 based DNA vaccine also induced humoral immunity against BVDV and prevented the infection (Young *et al*., 2005). Further, Liang *et al*. (2006) found that the DNA prime boost regimens were effective for preventing BVD in cattle when compared to the administration of DNA vaccine or subunit vaccine alone.

With the advent of recombinant DNA technology, VP1 gene based DNA vaccines are being utilized for developing effective vaccines against foot and mouth diseases (FMD) (Balamurugan *et al*., 2004; Dong *et al*., 2005), which is one of the most devastating and highly contagious diseases in cattle and swine and many wild cloven-hoofed animal species (Balamurugan *et al*., 2004; Grubman and Baxt, 2004). The causative agent *Aphthovirus,* belonging to the *Picornaviridae* family, is considered one of the fastest multiplying viruses. There are seven immunologically distinct serotypes: A, O, C, Asia 1, and SAT (Southern African Territories) 1, 2, and 3 (Ferris and Donaldson, 1992; Paton *et al*., 2005). The commonly observed manifestations in cattle are anorexia and fever, and vesicles on the tongue, dental pad, gums, lips, and on the coronary band and interdigital cleft of the feet (Barteling and Vreeswijk, 1991; Ferris and Donaldson, 1992). Vesicles may also appear on the teats and udder, particularly of lactating cows. Young calves may die before showing any vesicles because of virus-induced damage to the myocardium. Currently, for the prevention and control, FMD vaccines based on inactivated virus are used which are effective, but vaccinated animals exhibit serotype-specific immunity and only short-term protection from infection (Barteling and Vreeswijk, 1991; Paton *et al*., 2005). To counter this, DNA vaccination could be one of the most promising strategies, allowing a safe and efficient alternative to conventional vaccination. Plasmid DNA encoding the FMDV VP1 capsid protein followed by boosting with a VP1 peptide conjugate resulted in production of high titers of neutralizing antibodies (Shieh *et al*., 2001). A gene gun based FMD DNA vaccine encoding VP1 has also been developed by Benvenisti *et al*. (2001). Prime-boost strategy has exhibited its potency to enhance immune responses, which would be a key factor for the success of a suitable vaccine against FMD (Jin *et al*., 2005). It has been also suggested that use of interleukin-1 gene along with VP1 gene is capable in producing enhanced immune response against FMD (Shao *et al*., 2005; Park *et al*., 2006). Recently, a microparticulate based DNA vaccine has been developed and tested successfully, that code for the T and B cell epitopes of the FMDV (Wang *et al*., 2006).

Apart from the above mentioned diseases, researchers have tried to develop DNA vaccines against bovine rotavirus (BRV), bovine leukemia virus (BLV), rickettsial organisms and ectoparasites. A gene encoding the VP4 protein of BRV was found effective in stimulating a Th1-like immune response (Suradhat *et al*., 1997). Brillowska *et al*. (1999) reported that the plasmid with BLV envelope gene encoding glycoprotein gp51 and transmembrane glycoprotein gp30 has been found capable of generating an effective cellular immune response. Also, a DNA vaccine encoding the fusion (F) gene of bovine respiratory syncytial virus (BRSV) induced significant protection against BRSV infection in calves (Taylor *et al*., 2005). Nucleic acid vaccine, containing the gene of a major surface protein, MSP1b, of *Anaplasma marginale*, offered partial protection against challenge (de Andrade *et al*., 2004). In another study, DNA construct involving ORF of genes, cpg1, GroEL and GroES of *Ehrlichia ruminantium* could partially protect cattle against “heart water” disease (Simbi *et al*., 2006). The potential of DNA immunization with plasmid encoding antigen Bm86 to induce humoral and cellular immune responses against the ectoparasite *Boophilus microplus* has also recently been studied by Ruiz *et al*. (2007).

CONCLUSION

The role and capability of killed or live vaccines in preventing and controlling various diseases of domestic livestock has been well established. Still, a complete prevention or eradication of many infectious agents is a

distant dream. Moreover, the drawbacks of the conventional vaccines have forced the research fraternity to evolve newer alternatives, among which nucleic acid vaccines holds much promise. Vaccination with DNA is one of the most promising novel immunization techniques against a variety of bovine pathogens, for which conventional vaccination regimens have been less effective. The utility of DNA vaccines and their advantages have been proven beyond doubt. But the efficacy of this third generation vaccine largely depends on proper optimization and delivery. Various combinations of delivery systems and route of delivery can enhance immunity to DNA-based vaccines and make them practical for administration in large animals. After many years of experimentation, DNA vaccines have become well established in clinical trials done in experimental animal models. However, they have yet to proceed past the second phase trials. Thus, much promise is there regarding the development of safer, cheaper and effective DNA vaccines, taking into consideration of the various attempts currently taking place in the field of life science research. It is expected that in near future, research will focus to globally effect the implementation of potent DNA vaccines that are highly capable to address emerging and highly threatening infectious agents of bovines and other domastic animals.

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