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Review Article

Recent Advances in Vaccination Strategies and Future Perspectives for the Control of Newcastle Disease in Pakistan: A Review

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ABSTRACT

Newcastle disease (ND) is one of the most important viral disease affecting chickens, causing unbearable loses in both backyard and commercial chicken in Asia, America and Africa The mortality rate due to velogenic strain can be up to 100 % thus destroying the whole flocks A number of vaccination strategies have been implied to prevent this fatal disease in Pakistan but with a limited success Vaccination with the lentogenic strain invokes only humoral response while vaccination with the velogenic strains could be dangerous for the immuno-compromised birds Emerging concept of DNA vaccine may be a mile stone in the control of ND in Pakistan Better safety and satisfactory immuno-stimulatory profile augments the importance of DNA vaccine This review is a brief description of ND status in Pakistan, pros and cons of various vaccination approaches and recent advances in development of ND vaccines.

Key words: Cytokines, DNA vaccine, Newcastle, Poultry, Vaccination

INTRODUCTION

In this modern era with increasing rate of human population, availability of ad-libitum food to every human being is a big challenge. The increasing demand for food throughout the world is a point to ponder and is a cloud on the horizon for developing as well as developed countries For years poultry sector has been serving as a source of quality food throughout the world In order to raise to the occasion poultry production has been shifted from small rural flocks to highly advance commercial broiler and layer farming which helped a lot in filling the demand and supply gap around the globe.

The poultry industry in Pakistan is one of the leading industries and is a source of livelihood for more than 15 million people, contributing 238% of total meat production in Pakistan (Govt of Pakistan: Economic survey of Pakistan, 2009-2010). Poultry sector is progressing with an average rate of 8-10% annually (Ahmed *et al.*, 2009) Every family in rural areas and one out of five families in urban areas is involved in poultry production (Sadiq, 2004; Numan *et al.*, 2005). Poultry industry in Pakistan is affected by several factors including infectious diseases such as Newcastle disease (ND), an important poultry disease which is the most notorious cause of chicken mortality (Siddique *et al.,* 2005). Newcastle disease is a fatal and highly contagious viral disease of chickens, caused by ND virus (NDV) belonging to the genus Avulavirus of the subfamily Paramyxovirinae and family *Paramyxoviridae* (Al-Garib *et al.*, 2003), and is characterized by respiratory, digestive and nervous signs (Ahmad *et al.*, 2007). Birds infected with ND may die without showing any sign or may show various signs from mild air sacculitis to severe visceral and nervous involvement ultimately leading to paralysis and death of the birds (Abbas *et al.,* 2006).

Newcastle disease in Pakistan

In developing countries like Pakistan poultry plays an important role in rural and commercial economy. There are about 110591 million poultry birds in Pakistan (Khan *et al.,* 2011). According to economic survey of Pakistan 2011-2012 contribution of poultry meat out of total meat production is 258% and a growth rate of 8-10% annually

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(Govt of Pakistan: Economic survey of Pakistan, 2011-2012) Newcastle disease poses a huge threat to the poultry industry of Pakistan. In a report published in April, 2012 this virus was reported to kill 44 million broiler birds causing a loss of 6 billion rupees (The poultry site).

Despite of all the prophylactic efforts, including biosecurity and vaccination programs it is still causing outbreaks in layer, broiler and breeding flocks (Numan *et al.*, 2005; Abbas *et al.*, 2006). Newcastle disease is commonly found in both the unvaccinated as well as vaccinated flocks in Pakistan, severely affecting the production performance of both the broiler and layer flocks (Akhtar and Zahid, 1995). The annual incidence has been reported upto 38% in broiler and layer flocks (Anjum, 1990) and mortality within the flocks of commercial broiler chicken has been reported up to 50% (Siddique *et al.*, 1986).

The entire flock usually becomes infected with the virus within three to four days Virus is usually spread through contaminated fomites, clothing, shoes and free-flying birds It can also be transmitted through air but to a smaller distance (Shuaib *et al.,* 2006).

Reports showing the isolation of velogenic NDV from backyard poultry without appearing clinical signs revealed that these birds are carriers and continuously shed the virus hence introduction and spread of the virus may remain unnoticed (Munir *et al.,* 2012). This resulted into an endemic situation in Pakistan today Although strict biosecurity measures and strict vaccination schedules are practiced in commercial poultry farms but ND is still prevalent in such farms This might possibly be due to the difference between the vaccine and field strain of virus or due to defective replication machinery, as in case of most RNA viruses, causing rapid mutations and consequently strain (Cattoli *et al.,* 2011; Munir *et al.,* 2011).

Introduction of new NDV is one cause for disease out breaks in vaccinated poultry birds against which the poultry birds have very little or no immunity Poor manufacturing standards, application of expired vaccine batches, absence of proper storage facilities and vaccine handling during transportation are some factors which limit the efficacy of vaccines in developing countries like Pakistan (Vui *et al.,* 2002; Numan *et al.,* 2005).

Newcastle disease has been reported in studies conducted in different areas as one of the main agent causing respiratory distress (Ahmed *et al.,* 2009), and found that Velogenic NDV and Avian Influenza (AI) H9 isolates along with the secondary infection of Ecoli and salmonella were involved in the respiratory outbreak in the layer flocks of Sammundri, Gojra and Kamalia areas of Punjab province of Pakistan Prevalence of ND in local and fayoumi breeds of rural poultry in district Sheikhupura has been reported 4033% (Mustafa and Ali, 2005) It was found that 9807% broiler and 100% layer serum samples collected from Faisalabad and surrounding districts were found positive for specific immunity to NDV (Numan *et al.,* 2005). There is need to conduct such surveys in other areas where commercial poultry farming is practiced as well as in rural poultry to assess the status of ND in our country and adopt suitable strategies to control the disease.

Vaccination Strategies in Pakistan and Their Efficacy

Vaccination against ND is routinely done in most of Asian countries (Rehmani, 1996). Vaccination practice in Pakistan includes initial vaccination with LaSota or Mukteswar strain followed by the application of Mukteswar or Komarov strains Newcastle disease, virus strain F, is also used for vaccine production in Pakistan and has been reported to give very good results in day old poultry birds, when inoculated through nasal route Mesogenic strains are mostly used in older birds as a booster vaccine after lentogenic vaccine because these are considered unsafe for young chicks (Rehmani, 1996). However, respiratory signs, sometimes complicated with secondary bacterial infections are reported after use of these live vaccines Thus these vaccines may not suffice the need for satisfactory control of disease (Rehmani *et al.,* 1989). Mostly, out breaks of ND occur during winter season as increasing demands of eggs compel the farmers to vaccinate their laying flocks with Mukteswar or Komarov strain of ND vaccine as precautionary measure The pullets vaccinated with Mukteswar ND vaccine produced more eggs 715% than pullets vaccinated with Komarov strain ie 564% and non-vaccinated pullets ie 518%, however, peak egg production was 823% (Rehmani *et al.,* 1989).

Many studies have been done to attest the efficacy, potency and safety levels of Lasota ND vaccine in poultry birds (Akcadag *et al.,* 1984; Knezevic, 1989; Paulillo *et al.,* 1989; Reddy *et al.,* 1992) It has been reported that LaSota ND intraocular vaccination is superior to F strain ND vaccine and Mukteswar ND vaccine in Pakistan (Rehmani, 1996) and all commercial ND LaSota vaccines produced similar kind of immunity level in birds (Abbas *et al.,* 2006). However, different ND vaccines showed variable immune responses in birds when administered through water. It was observed that protection level of lentogenic F strain vaccine and Lasota strain vaccine was about 8590% while Mukteswar vaccine protection level was 45% when administered through drinking water (Kojnok *et al.,* 1977). It was also observed that intraocular vaccination produced more immune response as compared to drinking water route.

Level of protection in laying birds against ND can be improved by hyper immunization of the hens before laying and adopting better management conditions (Numan *et al.,* 2005; Siddique *et al.*,2005). Young chicks should be vaccinated before 12 days of age because delay in vaccination makes birds more susceptible to infection due to decline in maternal antibodies with the passage of time (Matuka *et al.,* 1976). Sufficient immunity is produced when birds are vaccinated at 12 days of age and this immunity is enough for the protection of infection until 7th week of age In this way reasonable antibody titer can be maintained by single shot of vaccine and there is no need to vaccinate birds twice in young age.

In one report, vaccination may lead to suppressed body growth and vaccinated birds gained less body weight and showed less feed conversion ratio as compared to non vaccinated birds It has also been reported that ND Lasota strain vaccine cause suppression in weight gain (Alexander *et al.,* 2004). However Lasota ND vaccine produces rapid immunity in young chicks (Shuaib *et al.,* 2006) Moreover, birds are more protected with the use of

Lasota as primary vaccine and Mukteswar as a second shot vaccine than Lasota vaccine alone No significant differences were observed in the haemagglutination inhibition (HI) antibody titers between LaSota followed by Mukteswar and LaSota alone vaccines, but a significant difference was found between the protective indexes of both schemes (Ahmad *et al.,* 2007). The factors which influence vaccination results in poultry birds include virus strain chosen for vaccine purpose, inhibitory response of maternal antibodies and ability of ND vaccine to induce stress on body Other reasons include enhanced secondary infections, poor vaccine quality, heat stress, water deprivation, contact of ND vaccine with viricidal chemicals like phenol or alcohol and birds with impaired immune response due to any reason can lead to vaccine failure (Tariq, 1999; Sil *et al.,* 2002; Vui *et al.,* 2002).

DNA vaccine; a promising tool to control Newcastle disease in Pakistan

Most of the outbreaks of ND in Pakistan are associated with velogenic pathotypes but to control the disease, both live attenuated and inactivated vaccines made from lentogenic and mesogenic NDV strains are used However live vaccines homologous to highly pathogenic velogenic pathotypes are not available as per recommendation of OIE; that does not allow the use of viral strains of intracerebral pathogenecity index (ICPI) values above 07 to be administered in day old chicks in order to avoid the unnecessary spread of velogenic pathotype (OIE, 2012). On the other hand Lasota, B1 and Clone 30 strains (lentogenic pathotype) used for vaccine production are significantly different from the velogenic pathotype and they may not provide optimum protection to birds against field virus (Munir *et al.,* 2012). Moreover, such vaccines are not enough to prevent the viral shedding when use of live attenuated vaccine is in practice Thus although vaccination is a tool for the prevention of clinical disease, however, it's not enough to prevent the shedding and spread of diseased virus (Miller *et al.,* 2009).

The new concept of immunization through DNA vaccine first came in the early 1990's, when it was found that intramuscular injection of a recombinant plasmid DNA resulted in the expression of a gene in mice (Wolff *et al.,* 1990). This led to the start of nucleic acid based immunization approach The DNA vaccine generated, using recombination of a pathogen's immunogenic gene and an optimized bacterial plasmid, is a novel approach that could support the efforts made to the development of newer immuno-prophylactics for controlling infectious diseases of poultry These third generation vaccines are having many advantages when compared to the conventional inactivated or live vaccines Induction of both cellular and humoral immunity with superior long lived cellular immunity, and multivalent vaccine providing immunity against a variety of pathogens and non dependency on cold chain, are few among those advantages (Dunham, 2002; Song *et al.,* 2005; Meeusen *et al.,* 2007).

For developing DNA vaccines, generally the gene encoding immunogenic protein of a pathogen which is protective to host is incorporated into a suitably designed plasmid which has been derived from microbial agents This gene encoded plasmid DNA, when administered to

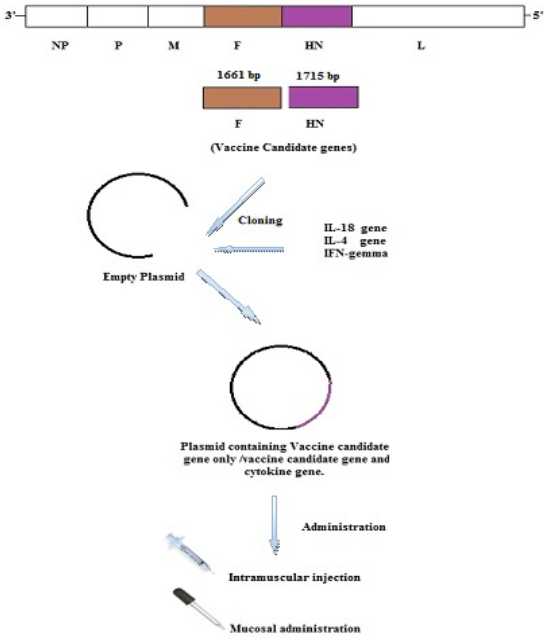

Fig 1: General concept of DNA vaccine against Newcastle disease.

host, is capable of getting transcribed and translated into a peptide within the host cells to generate protective responses on encountering with the host immune cells.

The ND virus has a single-stranded negative-sense RNA genome of 15 Kbp containing six genes which encode proteins including nucleoprotein (NP), phosphoprotein (P), matrix protein (M), fusion protein (F), hemagglutinin-neuraminidase (HN), and polymerase protein (L) Virulence and interaction to cells is mainly controlled by F protein, HN protein and envelope glycoproteins (Nagai,1993; Stone-Hulslander and Morrison, 1997; McGinnes *et al.,* 2002; Gravel and Morrison, 2003) Both the F and HN proteins have been targeted for vaccine development (Sakaguchi *et al.,* 1996). Several studies conducted in specific-pathogen-free (SPF) chickens have shown that both F and HN are also able to produce protective immune response in SPF chickens (Heckert *et al.,* 2002; Loke *et al.,* 2005; Chaturvedi *et al.,* 2011) Incorporation of the cytokine genes like IL-18 and IL-4 etc provide an adjuvant effect thus enhancing the efficacy of DNA vaccine Figure 1 summarizes the general concept of DNA vaccine against the ND.

Various approaches used in Development of Newcastle Disease DNA vaccine

Linear plasmid expressing the F gene of virus has shown to protect the chicken against devastating ND (Sakaguchi *et al.,* 1996). Co-administration of plasmids expressing both HN and F protein of the virus are better for the induction of antibody response and give superior protection against viral challenge than either component alone (Loke *et al.,* 2005).

Recombinant chicken IL-18 co-expressed with ND-HN protein (rHN/chIL-18) in fowl pox virus is reported to potentiate the cell mediated immune response (Su *et al.,* 2011). Vaccination of chicks at 2 weeks of age with various dilutions of rHN or rHN/chIL-18 emulsified in

Table: Various approaches used for development of Newcastle Disease DNA vaccine

	Approaches for development of NDV-DNA vaccine	Reference
1)	Linear plasmid expressing the f gene of NDV	(Sakaguchi *et al.,* 1996)
2)	Co-administration of plasmids expressing both HN and F protein	(Loke *et al.,* 2005)
3)	Adjuvant effects of chicken interleukin-18 in avian Newcastle disease vaccine (prokaryotic cell-expressed full length and mature chicken IL-18)	(Hung *et al.,* 2010)
4)	Recombinant chicken IL-18 co expressed with Newcastle disease HN protein in fowl pox virus	(Su *et al.,* 2011)
5)	Plasmid expressing HN,F and cytokine genes (IFN-gemma and IL-4 genes)	(Sawant *et al.,* 2011)

mineral oil and later on administration of booster dose two weeks after priming produced a range of immune response in birds when an antigen challenge was given, 28 days post priming Experimental results have shown that the protection level against NDV challenge was 100% in the birds receiving rHN with rchIL-18 emulsified in mineral oil as compared to the birds receiving only rHN in mineral oil (67% protection). No protection was observed in the chicken receiving only rHN. It also emphasizes the need of mineral oil to stimulate an optimum immune response as mineral oil slowly releases the antigen providing better antigenic stimulation over longer period of time (Aucouturier *et al.,* 2001; Petrovsky and Aguilar, 2004). The protection level is related to the induction of IFN gemma and cell mediated immune responses However, a dose dependent effect of rHN when used with chIL-18 was observed with low level of humoral response when the quantity of rHN was below 50ng but the protection level was yet unaltered due to activation of cell mediated immune response (Su *et al.,* 2011) as low level of antigen preferentially stimulate T cell respone (Mingxiao *et al.,* 2006) Various approaches used in development of ND-DNA vaccine are summarized in Table 1.

Conclusion

The vaccines currently being used to prevent ND are not able to control the disease and sporadic outbreaks are reported every year even disease remains endemic throughout the year The DNA vaccines are coming up as promising technology for the induction of antigen specific immune responses and therefore much attention has gone into improving their immune potency (Oshop *et al.,* 2002; Chaturvedi *et al.,* 2011). Hyper-immunization of birds using immune potentiating agents like cytokines can be a proficient approach to cope with the situation Therefore, the introduction of efficient vaccination strategies is needed in order to rise to the occasion.

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