

EVALUATION OF EFFICACY OF KILLED AND COMMERCIALY AVAILABLE LIVE NEWCASTLE DISEASE VACCINE IN BROILER CHICKENS IN PAKISTAN

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ABSTRACT

The study evaluated the efficacy of Newcastle Disease killed and commercially available live vaccine. Moreover, study also highlighted the inappropriate use of Newcastle Disease live vaccine as a booster dose providing the space to virus for its growth and multiplication in semi controlled broiler farms creating threat to whole poultry industry. Serum antibodies level was assessed by Haemagglutination Inhibition method and efficacy of Newcastle Disease killed and live vaccine was also determined using challenge test. Group "A" immunized with killed vaccine using homologous strain provided 90% to 100% protection. However, group "B" immunized with Newcastle Disease live vaccine (Heterologous strain) provided protection not more than 60% when challenged with velogenic viscerotropic form of Newcastle Disease Virus isolated from the recent outbreak. The results of the present work concluded that oil emulsified killed vaccine provided good protection using indigenous isolate against Newcastle Disease. Regression analysis showed highly significant ($P < 0.001$) correlation between antibodies titre against Newcastle Disease versus age of the flock in group immunized with killed vaccine and significant ($P < 0.01$) correlation in that group immunized with live vaccine.

Key words: Newcastle disease, killed vaccine, commercial live vaccine, broiler.

INTRODUCTION

Livestock sector is developing day by day with growth rate 3.70% and in it poultry sector share is 10.4%. However, poultry sector growing 7-8% and poultry meat is 26.8% of total meat production in Pakistan (Economic Survey of Pakistan, 2012-13). In Pakistan, commercial poultry farming is still confronted with many viral as well as bacterial problems, which are hindering its progress (Saima *et al.*, 2010). First time disease was reported from Newcastle, England in 1926, therefore known as Newcastle Disease (Xiao *et al.*, 2012) and then in India from Ranikhet in 1927, hence called Ranikhet (Chakrabarti, 2007). Newcastle Disease virus (NDV) is a worldwide distributed virus, which is the sole member of Avian Paramyxovirus type 1 (APMV-1) of genus Avulavirus, subfamily Paramyxovirinae and family Paramyxoviridae and it affects almost all species of wild as well as domestic birds (Susta *et al.*, 2010). It is well documented that sequence of amino acids at cleavage site determines the range of protease enzymes that cuts the cleavage site and the fusion protein is activated which indicates the pathogenicity of virus (Smietanka *et al.*, 2006). Vaccines are used to prevent and control the infectious disease in poultry industry. Therefore, a proper stringent vaccination schedule must be applied to minimize/eradicate the emergence of clinical disease at farm level (Marangon and Busani, 2006). However, now a days, ND vaccination includes the use of killed and

attenuated live vaccines to induce protection against contagious diseases. Additionally, mutation changed the circulating field virus after a period of time and making this phylogenetically different from vaccine strain that reduced the efficacy of ND live vaccine. Then, to improve the efficacy of commercial vaccine, new vaccines candidates must be investigated in the laboratories (Rauw *et al.*, 2009). Even with widespread use of different types of commercially available attenuated live and inactivated vaccines, ND is still a major threat to the poultry industry in developing countries including Pakistan. Currently ND is widely present in all areas of Pakistan and outbreaks have been increased and reported 6 billion US dollars losses during last winter outbreaks i.e. November 2011 to March 2012. Variation in broiler disease pattern might be due to high intensification as well as industrialization of poultry industry. Therefore, present study was conducted to evaluate the efficacy of commercially available live and ND killed vaccine, using homologous isolate of viscerotropic velogenic form of NDV, collected from the recent outbreak.

MATERIALS AND METHODS

Newcastle disease virus (Indigenous isolates) is being used for oil emulsified vaccine preparation used in the experimental trial. The sample was collected from the recent outbreak and confirmed by post-mortem

examination as well as by molecular method (Reverse Transcriptase-Polymerase Chain Reaction). Infectivity titre of the virus was assessed by making tenfold dilutions and inoculating each dilution into five eggs. Titration results revealed that the Egg Infective Dose 50 (EID₅₀) of NDV was 10^{9.16} EID₅₀ per ml, calculated by Spearman and Karber Method (1974). Nine day old embryonated chicken eggs were inoculated with ND virus with dose rate 0.2ml per egg. Before inactivation, Amnion allantoic Fluid (AAF) centrifuged at 4,000 rpm (Hettich Zentrifugen, Germany) for 10 minutes and supernatant was separated and treated with formalin (37.5%) with dose 0.05%. Thimerosal was also used to stop the bacterial as well as fungal growth with dose rate 0.01% in the AAF. The fluid was mixed thoroughly in an Orbital Shaking Incubator (Model # PA-H-42/250) at 37°C for 24 hours. Inactivated virus sample was inoculated for at least two consecutive times into specific pathogen free eggs to confirm complete inactivation assessed by spot haemagglutination test. The inactivated antigen emulsified in Montanide oil (ISA 70M VG) (Seppic, France) used as an adjuvant to obtain water in oil emulsion vaccine. Equal volume of Montanide oil was taken in a sterile beaker and inactivated antigen was added drop wise and mixed with laboratory homogenizer (T25 basic ULTRA-TURRAX, IKA (Germany)) continuously at lowest speed. Then it was mixed at speed 17.500 for 2 minutes and 20.500 for 30 seconds for proper mixing and stable white (water in oil) emulsion was prepared. Sterility, safety and stability of the prepared vaccine were checked and confirmed prior to conduct the trial.

Experimental Design: The present study was conducted on day-old (n=80) broiler chicks (*Gallus domesticus*) along with a semi controlled broiler flock (n=7000) procured from same hatch of commercial hatchery, having an average body weight 40gm per chick, during April 2013 to June 2013. Rearing of chicks was arranged in a semi-controlled shed located at 23Km away from Muridke (Northern Punjab) Pakistan. Additionally, Commercial Environmentally Controlled Houses used a different strategy of vaccination than the semi controlled Houses. Other parameters like Feeding, Medication and Environmental conditions were similar in both flocks. After four days of acclimatization, birds were divided randomly into three groups. Group A (n=40) and B (n=7000) as an experimental groups and the vaccination schedule used, shown in Table-1. Group C(n=40) was kept as a control.

Serology: Prior to vaccination blood samples were collected on random basis from 10 birds to evaluate their maternal immune status. After that eight birds from each group (A, B and C) were randomly selected to collect blood samples on 14th, 21st, 28th, 35th and 42nd day of age respectively. Samples were labeled during blood

collections and sera were drawn from each sample and labeled and stored at -20°C till further processed. The sera were used to assess antibodies level in blood by a conventional diagnostic method, Haemagglutination Inhibition (HI) test. HI titre was recorded of each sera sample and Geometric Mean titre (GMT) values were calculated following Brugh, (1978).

Challenge Virus: Birds were challenged at 28th and 42nd day of age respectively with ND isolate (CK/NDV/KSUR/PK/ 2011/55) characterized as velogenic at Quality Operation laboratory, University of Veterinary and Animal Sciences Lahore, used as a challenge virus in this study. The virus challenge dose was 10,000 units of CLD₅₀ (10^{4.5})/ml/bird calculated according to Spearman Karber Method (1974). Eye drop method was used to challenge the birds and kept under observation for 15 days for development of any abnormality or mortality.

Data Analysis: In statistical analysis, Geometric Mean Titres were calculated following Brugh (1978). Furthermore, correlation coefficient and regression analysis were performed to investigate the level of significance among given variables by using MS Excel (SPSS software) and interpretation was done according to Zar, (1996).

RESULTS AND DISCUSSION

The results of current study revealed that the maternal antibody titre at the age of 5th day was 4.1, evaluated using haemagglutination inhibition test, diagnostic method for Newcastle Disease. The decline in antibody titre was recorded at 14th day of age and the reasons of this decline was explained by Tizard, (2004) that decline in antibodies titre occurred due to neutralization of the vaccine virus by circulating maternal antibodies. The HI titre against ND using killed ND vaccine as a primary and booster dose started to increase at 21st day and recorded at peak at 28th day with a significant difference in vaccinated and non-vaccinated group. These results showed that the use of ND killed vaccine as a primary and booster dose induced efficient and long lasting immunity, sustained up to marketable age of broiler chicken. These findings are in agreement with World Organization for Animal Health, (2012) notification that killed ND vaccine induces effective and long term immunity than that of live ND vaccine (LaSota). Quite a few references are available that inactivated vaccine of ND gave better protection than live ND vaccine (Rehman *et al.*, 2002) as well as slow release of antigen provide high and long term immunogenicity against ND (Foliste *et al.*, 2007). Regression analysis was also applied and results revealed that age of the flock has highly significant (P< 0.001) positive correlation with GMT values of antibodies in serum against ND in group "A" (Table-1.1). In group "B", a routine vaccination

schedule of semi controlled houses was adopted to compare immunity status of flocks vaccinated with commercially available ND live (LaSota/ND Clone) and killed vaccine i.e. prepared using indigenous isolate collected from recent ND outbreak. Vaccination at 7th day revealed that decline pattern of ND titre was almost similar as in other group and then the titre raised up to 3.0 (\log_2). Moreover, maximum titre (6.0) was recorded at 28th day when flock was boosted at 18th day of age. But the antibody titre showed a sudden decline up to 3.8, when recorded a week later after the third booster dose with commercially available ND live vaccine (ND Clone). Therefore, it is advisable that booster dose should be given until and unless already existing antibodies titre drops to a certain level where it will not interfere with the priming antigen (vaccine) (Ahmad *et al.*, 2007). Furthermore, minimum interference to maternal immunity i.e. 0.5 (\log_2) was recorded with ND killed vaccine while using ND live vaccine interference was maximum i.e. 2.1 (\log_2) (Fig-2.3). It was concluded that killed vaccines had minimum adverse reactions to maternal immunity than live vaccines. According to the results in this study level of circulating antibody response for long duration in control group was also recorded as mentioned earlier by (Waheed *et al.*, 2013). The factors contributing in ND outbreaks like poor quality of vaccine and its lack of a cold chain system cannot be overruled (Abolnik *et al.*, 2004; Bogoyavlenskiy *et al.*, 2009; Hassan *et al.*, 2010; Ke *et al.*, 2010) along with inappropriate vaccination schedule against ND. Dortmans *et al.* 2012 also reported that vaccine failure is due to inadequate vaccination practices results in poor immune status of flocks, providing the space to virus for their growth and multiplication in field. Regression analysis showed significant ($P < 0.01$) correlation between GMT values of ND versus age of the flock vaccinated with ND live vaccine in group "B" (Table-2.1). Vaccine efficacy was evaluated using challenge test and results revealed

that ND killed vaccine prepared using homologous isolate provided 90-100 percent protection. Expert's investigations revealed that killed vaccine homologous to circulating field virus not only protect broiler from mortality and morbidity but also significantly reduce viral multiplication and its shedding (Miller *et al.*, 2013). Furthermore, group "B" provided unsatisfactory protection 50% and 60% when challenged with vvNDV at 28th day of age and then at the marketing age (i.e. 42nd day) respectively. More or less similar findings reported by Hu *et al.* (2010) when they investigated and found flocks positive for ND despite the flocks were regularly vaccinated with LaSota strain (ND live vaccine). Furthermore, phylogenetic analysis results revealed that these isolates were not related to the vaccine strain (LaSota). However, unvaccinated birds (Group "C") were highly susceptible to challenge virus and 100% deaths were recorded and mortality was examined by post mortem examination and gross lesions were recorded. Moreover, pin point hemorrhages were observed in control group only. Surprisingly, nervous signs with torticollis and circling movement in 10% chickens were recorded within two weeks of post challenge (Group "B"). These results revealed that for somehow the commercial attenuated live ND vaccine cannot provide satisfactory protection against velogenic form of ND. Similar findings also reported by Yu *et al.* (2001) that vaccine strain LaSota/46 provided protection not more than 40% when challenged with ND strain isolated from recent outbreak. Furthermore, this failure of immunization with LaSota (lentogenic) strains is due to fundamental differences in the peptide patterns of vaccinal strain and challenging field strain (Khan *et al.*, 2005). Same line of findings also reported by Miller *et al.* (2007) that vaccines prepared from homologous isolates, phylogenetically closer to field circulating virus may provide efficient protection against ND by decreasing virus shedding from infected birds.

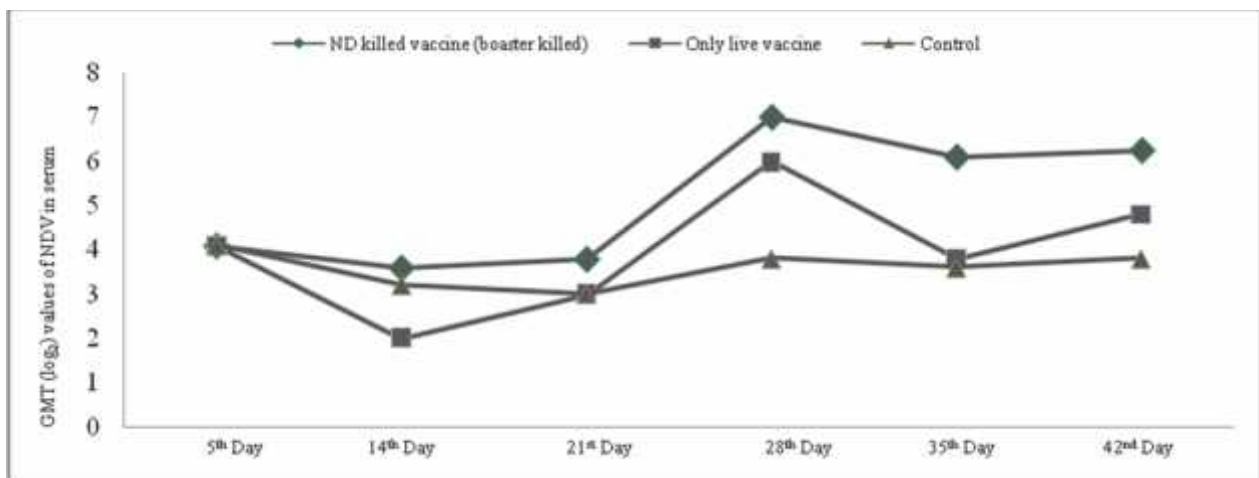


Fig-2.3: Comparison of ND inactivated and live vaccine in decrease in maternal immunity

Table 1. Experimental design with vaccination schedule of different groups

Day of vaccination	Group A	Group B	Group C
5th day	ND killed vaccine (S/C)		
7th day	ND-IB* vaccine (D/W)	ND-IB* vaccine (D/W)	
10th day	IBD* vaccine (D/W)	IBD* vaccine (D/W)	IBD* vaccine (D/W)
18th day (Booster dose)	ND killed vaccine (S/C)	ND-Clone* (D/W)	
28th day		ND-Clone* (D/W)	

*Commercially available vaccines i.e. ND+IB (Bivalent) and ND-Clone (Monovalent)

*IBD= Infectious Bursal Disease, IB= Infectious Bronchitis, S/C = Subcutaneously,

D/W= Drinking Water

Table 1.1. Correlation GMT values of ND versus age of flock when vaccinated with inactivated ND vaccine as a primary and booster dose

Statistical parameters of different relationships among GMT values of ND versus age of the flock, probabilities (p), n = 30 in each case.

Relationships	Correlation coefficient (r)	Intercept (a)	Regression coefficient (b)	Standard error of b (S.E.)
Age of flock, (x) GMT of ND (inactivated ND vaccine), (y)	0.7854***	2.5554	0.1204	1.2236

***P < 0.001

Highly significant

Table 2.1. Correlation GMT values of ND versus age of flock when vaccinated with commercial live attenuated ND vaccine

Statistical parameters of different relationships among GMT values of ND versus age of the flock, probabilities (p), n = 30 in each case.

Relationships	Correlation coefficient (r)	Intercept (a)	Regression coefficient (b)	Standard error of b (S.E.)
Age of flock, (x) GMT of ND (live attenuated ND vaccine), (y)	0.5048**	2.6055	0.0728	1.6063

**P < 0.01

Significant.

Conclusion: Inactivated ND vaccine with stringent vaccination schedule is more effective than attenuated live to minimize/eradicate ND viral shedding as well as its multiplication. Moreover, homologous strain use for immunization is more efficient as it provided 90 to 100 percent protection and minimum adverse reactions to maternal immunity than live vaccines that provided unsatisfactory protection 50 and 60 percent at 28th and 42nd day of age respectively.

Acknowledgement: Authors would like to thank Mr. Abdullah and Sons for their cooperation, allow us to conduct experimental trial on their farm.

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