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Research Article

EVALUATION OF DIFFERENT ROUTES OF VACCINATION BY CLONE VACCINE ON HUMORAL ANTIBODY RESPONSE

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ABSTRACT: Prevention of Newcastle disease in broiler birds is a priority for successful poultry industry. The present study evaluates the immune response to Clone 30 live vaccine alone and in combination with inactivated vaccine administrated by different routes in broiler chickens. To evaluate the various route of vaccination and inactivated vaccines on antibody response, Clone vaccine was administered in different routes such as eye drop, spray, and drinking water with or without inactivated vaccine. Hemagglutination inhibition (HI) and enzyme-linked immunosorbent assay (ELISA) methods was used to assess the antibody response. Results indicated significant difference among different groups and the antibody titer showed highest in eye drop with inactivated vaccine group in both the tests at 42 th. day.

Key words: Newcastle disease, Vaccination route, Clone 30 vaccine, Inactivated vaccine, Antibody titer.

INTRODUCTION

Newcastle disease (ND) is a highly contagious and wide spread viral disease of the avian species causing severe economic losses in domestic poultry, especially in chickens (Al-Garib *et al.* 2003). ND is one of the most important diseases of poultry Globally ND remains as a major barrier to international trade in poultry and poultry products (Balachandran *et al*. 2014). Researchers indicated that the ND has most economical impact on the world than other viral disease (Alexander *et al.* 2008). Despite the advances in vaccines and vaccination programs in control of ND, the disease remains a major constraint in industrial poultry production worldwide (Alexander *et al.* 2012).

Various factors influence the outcomes of a vaccination programs in broiler industry, such as efficacy of the vaccine strain, the inhibitory effects of maternal and residual active immunity, the ability of the vaccine virus in antibody production, and secondary reactions that were due to vaccine strain or unsuitable route of vaccination (Lim 2014). An advantage of the live vaccines is that they can be administered at large scale. The method more popular for administration is by supply in the drinking water, although aerosols and eye-drop methods are utilized (Landman *et al.* 2017).For success in ND prevention, it is necessary to compare the available vaccine strains and efficient methods of application (Cardenas Garcia *et al.* 2014).

It was reported that the vaccination with live vaccines based on less virulent strains sometimes cause disease and growth retardation; therefore mostly the least or avirulent strain of the virus was use for live vaccination of poultry. Although this strategy reduces the vaccination reaction, but sometimes vaccination could not effective in preventing infection and transmission of virus to other birds (Burridge *et al.* 1975, Kapczynski and King 2005, Senne *et al.* 2004).

Currently mesogenic, lentogenic and a pathogenic enteric types of vaccine in use in worldwide (Swayne *et al.* 2013), but in Iran only lentogenic (includes: Hitchner B1, VG/GA, Cloned La Sota and La Sota) and a pathogenic enteric types (includes: PHY.LMV.42 and Ulster 2C) are used in ND prevention programs.

Vaccination of broiler chicken flocks against ND usually carried out by non-virulent live virus administered by spray or eye-drop or via drinking water. The various ways of administration usually produce considerable variation in the antibody responses of vaccinated birds, which causes variation in the levels of protection of broilers against the disease (Senne *et al.* 2004, Landman *et al.* 2017). It has been reported that simultaneous vaccination with live and killed ND vaccines resulted in better antibody response and protection (Lima *et al.* 2004). Following parenteral vaccination by an inactivated vaccine, the immune response is mostly humoral and is generally protective (Alexander *et al.* 2008). But the types

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of vaccines and vaccination programs vary widely (Alexander *et al.* 2008). Coarse spray is a practical alternative to eye-drop vaccination method in commercial poultry farms. Because the deposition of vaccine virus in the lower respiratory tract can cause adverse vaccinal reactions, hence these methods are intended to target only the upper respiratory tract (Landman *et al.* 2017, Landman *et al.* 2015).

Various vaccination programs comprise of simultaneously inoculating with live and inactivated vaccine to improve antibody production and better immune response against challenge viruses.

The objective of this study was to compare the antibody titers produced by Nobilis Clone 30 live vaccines alone and in combination with inactivated vaccine administrated by different routes in broiler chickens.

MATERIALS AND METHODS

Four hundred and twenty numbers of day old Ross 308 chicks was distributed randomly in 7 groups, each with 3 replicate of 20 chicks. Rearing conditions like ventilation watering and feeding were uniform in all groups. The birds were fed with a common diet formulated by Aviagen Co.

To evaluate the efficacy of various routes of vaccination and inactivated vaccines on antibody response, Clone 30 vaccine was administered in different routes, *viz.* as eye drop, spray, and in drinking water with or without inactivated vaccine (inactivated vaccine was administrated subcutaneously).

Vaccination performed on day 1, 14 and 30 by different routes in various groups. The last group was kept as control and only received normal saline as placebo. Hemagglutination inhibition (HI) and enzyme-linked immunosorbent assay (ELISA, IDEXX laboratories) methods were used to assess the antibody response of birds on day 13, 29 and 42 of vaccination. For this purpose in each group 18-blood samples taken, sera separated and sent to microbiological laboratory of Tabriz Islamic Azad University. Antibody titer against ND virus was assessed by ELISA kit instructions. The HI test was carried out according to the standard procedure described earlier (Majiyagbe and Hitchner 1977, Kaleta and Siegmann 1971).

Statistical analysis: The SPSS statistic software version 22.0 used for analysis of data, and One-way Analysis of Variances along with Duncan post hoc test used for evaluation of results.

RESULTS AND DISCUSSION

Results of HI antibody tests indicated there was significant difference among groups on day 13 of study ($p<0.05$). On day 29 antibody titers were different between control and all other groups ($p<0.01$), and spray + inactivated groups had the highest antibody titer. At the end of study, live vaccine used along with inactivated vaccine yielded higher antibody titers than the live vaccine alone. The results indicated that the eye-drop method with inactivated vaccine had highest antibody titers (Table 1).

Results of the ELISA test indicated that at day 13 the antibody titer was highest in spray + inactivated group, which was significantly higher than drinking water and control groups ($p<0.05$). On day 29 antibody titers were significantly different between control and all other groups ($p<0.01$), and the highest antibody titer was obtained in spray + inactivated group. At the end of study, the antibody titer in control group was significantly less than in experimental groups; also drinking water groups was significantly different from other vaccinated groups ($p<0.05$)). The highest antibody titer was observed in eye-drop + inactivated and next is spray + inactivated groups which were different statistically from live vaccine groups ($p<0.05$) (Table 2).

Newcastle disease is a major threat to poultry industry all around the world, and causes enormous economic loss at global scale. In developed countries outbreaks of ND and implementation of its control measures, including vaccination, are very costly for the poultry industry (Gue'ye 2002). Failure of vaccination or improper vaccination causes ND outbreaks widely. Generally, good vaccination program provides relative protection against the disease and mortality, but transmission of ND virus may continue in vaccinated flocks.

To prevent spreads of the disease more than 85% of the birds should have high level of antibody titer ($\log_2$ titer ≥3) following vaccination (van Boven *et al.* 2008).To obtain high level of protective antibody titers the vaccine content, vaccination program and administration method play important role (Van Boven *et al.* 2008).

Live vaccines stimulate local immunity and confer quick protection. However, there are some disadvantages in use of the live vaccines like presence of residual virulence. Many strains of live vaccine seem capable of causing low-grade disease or lowering growth rate of the vaccinated stock. This is particularly important for vaccine virus delivered by spray or aerosol method, which causes respiratory disease and even deaths (Lim 2014).

In spray method of vaccination a slight but significantly higher mean HI NDV serum titer is obtained, likely due to the presence of vaccine virus loaded respirable droplets or in the spray (Landman *et al.* 2017).

Administration of ND vaccines by intraocular and

Table 1. HI antibody titers against the Newcastle disease by HI test on different days.

Vaccination	HI Results		
	Day 13	Day 29	Day 42
Eye-drop + Inactivated	5.20±0.20^{*b}	5.70±0.25^{bc}	6.64±0.18^{c}
Eye-drop	4.70±0.20 ^{ab}	5.28±0.18^{bc}	5.00±0.31^{b}
Spray + Inactivated	5.24±0.34 ^{b}	6.14±0.38^{c}	6.40±0.24^{c}
Spray	4.96±0.04 ^{b}	5.33±0.18^{bc}	5.00±0.18^{b}
Drinking water + Inactivated	4.70±0.20 ^{ab}	5.68±0.24^{bc}	6.02±0.32^{c}
Drinking water	4.40±0.24 ^{ab}	5.03±0.42^{b}	4.57±0.20^{b}
Control	4.00±0.44^{a}	2.40±0.24^{a}	1.60±0.24^{a}
p value	0.031	0.001	0.001

*Different alphabetic indicate significant difference ($p<0.05$).
The results are as Mean ± SE.

Table 2. Serum antibody titer against Newcastle disease as measured by ELISA.

Vaccination	ELISA Results		
	Day 13	Day 29	Day 42
Eye-drop + Inactivated	13964.20±671.140^{c*}	14632.20±685.57^{b}	15970.60±527.26^{d}
Eye-drop	12375.20±614.33^{bc}	14161.80±594.65^{b}	13588.40±505.72^{c}
Spray + Inactivated	14037.80±490.32^{c}	15422.00±296.39^{b}	15387.20±427.76^{d}
Spray	12905.20±477.71^{c}	14243.60±584.02^{b}	13623.80±883.29^{c}
Drinking water + Inactivated	12415.00±862.54^{bc}	14262.40±636.46^{b}	15108.00±213.13^{cd}
Drinking water	10885.40±291.58^{ab}	13764.40±529.93^{b}	11561.20±433.94^{b}
Control	10069.40±179.40^{a}	6476.20±480.27^{a}	4260.00±328.92^{a}
p value	0.001	0.001	0.001

*Different alphabetic indicate significant difference ($p<0.05$).
The results are as Mean ± SE.

drinking water route resulted in highest titer of HI antibodies while administration of the vaccine through drinking water produced lowest titers of HI antibody (Rehmani 1996). Immune response in drinking water route of vaccination depends on the strain of vaccine. It was demonstrated that the F strain and La Sota strain produce 85.90% protection against challenge whereas the Mukteswar strain only provide 45% protection. Environmental conditions and age of birds affects the serologic response to drinking water method and to prevent from vaccine inactivation the vaccine containg water should be drunk as soon as possible (Rehmani 1996).

Earlier studies have reported that serologic response to primary vaccination with different strains of ND vaccines were similar but after second booster vaccination some strains produce better antibody response (Roy *et al.* 1998). Different live and inactivated vaccines used for prevention of ND in Iranian poultry farms, which confer only partial protection against the disease.

Inactivated NDV vaccines stimulate higher antibody titers in poultry and help to protect birds from morbidity and mortality; higher level of antibodies could decrease virus shedding and number of infected birds (Miller *et al.* 2013). Researchers indicated that the various live ND vaccines produce similar antibody responses but inactivated ND vaccines produce different levels of protections (Lin *et al.* 1990). The main disadvantage of the spray route of vaccination is the uncontrolled deposition of droplets in the respiratory tract of the birds and the high vaccine virus loss mainly due to evaporation (Landman *et al.* 2017).

It was indicated the most effective route for vaccination against ND was aerosol, followed by the intraocular method; intra tracheal administration or subcutaneous inoculation led to a marginal response (Eidson and Kleven 1976).The aerosol route of vaccination produce the highest levels of antibody, despite the lesser amount of vaccinal virus (Beard and Easterday 1967).

It was indicated that vaccination by the ocular route

protected birds from disease caused by virulent NDV (Degefa *et al.* 2004). Study in Ethiopia indicated booster immunization fully protected chickens from overt clinical disease by challenged virulent ND virus (Van Der Goot *et al.* 2007). Simultaneous administration of live and inactivated ND vaccine provide better protection against virulent NDV and successfully used to control of ND in poultry farms all around the world (Senne *et al.* 2004).

Our results showed that there was significant difference in serum antibody titer produced by the live vaccine along with inactivated vaccine in comparison to live vaccine alone, thus inactivated vaccine administration to broiler chicken were recommended. In addition, our results indicated that the antibody response in the eye and spray routes of vaccination was highest, both in HI and ELISA. Because of probable vaccination reaction in spray method it seems eye drop vaccination along with inactivated vaccine could be effective and may be recommended if there is no predisposing factor such as bacterial infections in birds are present.

The results indicated that Clone vaccine administration as eye drop, in combination with inactivated vaccine yielded the best antibody response and have the potential to reduce complication and losses from the disease.

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