

Evaluation of Different routes of vaccination by Avinew vaccine on humoral antibody response by HI and ELISA method

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Abstract

Newcastle disease is an important economical disease in broilers, therefore its prevention very important. The aim of this study was to compare the antibody titers produced by Avinew live vaccines alone and in combination with inactivated vaccine administrated by different routes in broiler chickens with HI and ELISA test. Four hundred and twenty day old Ross-308 chicks was distributed randomly in 7-groups, with 3-replicate. To evaluate the various route of vaccination and inactivated vaccines on antibody response, Avinew vaccine used in different ways include eye drop, spray, and drinking water with or without inactivated vaccine. Vaccination performed on days 1, 14 and 30 by different routes in various groups. Hemagglutination inhibition (HI) and enzyme-linked immunosorbent assay (ELISA) methods was used to assessment of antibody response at days 13, 29 and 42. Results of HI antibody tests indicated there was not any significant difference between groups on day-13 of study, but in ELISA the antibody titers was highest in spray + inactivated group ($p < 0.05$). At day 29 HI and ELISA antibody titers was different between control and all other groups ($p < 0.01$). At the end of study in all groups which live vaccine was used along with inactivated vaccine antibody titers was higher than live vaccine groups. The results indicated that the spray method with inactivated vaccine has highest antibody titers. The results indicated that the spray vaccination along with inactivated vaccination has highest antibody titers in both ELISA and HI method.

Keywords: Newcastle disease; Vaccination routes; Avinew vaccine; inactivated vaccine; live vaccine

Introduction

Newcastle disease (ND) is a highly contagious viral disease of various wild and domestic avian species (Seal *et al.*, 2000). Due to high susceptibility of domestic poultry and severe outcomes of disease in poultry farms, it has notable impact to poultry industry. It was reported that the ND has most economical impact on the world than other viral disease (Alexander *et al.*, 2008). Despite the vaccination in poultry population, ND outbreaks causes severe economic losses in poultry farms, the wide losses from poultry farms was reported from Netherlands in 1992 to 1993, UK in 1997, and USA in 2002 (Alexander *et al.*, 2008). Despite the advances in vaccines and vaccination programs in control of ND, the disease has negative impact on poultry production worldwide (Alexander *et al.*, 2012). During 2006 to 2009 ND, rabies and bovine tuberculosis was the most widespread animal diseases (Kapczynski *et al.*, 2013).

Vaccination could decrease clinical signs and mortality (Alexander *et al.*, 2008; Kapczynski and King, 2005). It was reported that the vaccination with live vaccines based on non-virulent virus strains causes disease and reduced growth retardation, therefore mostly the less virulent strain vaccine was used for vaccination of poultry. Although this strategy reduces the vaccination reaction, but sometimes vaccination could not be effective in preventing infection and transmission of virus to other birds (Kapczynski and King, 2005; Senne *et al.*, 2004). 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 disease outbreaks (Senne *et al.*, 2004). It has been reported that simultaneous vaccination with live and killed ND vaccines results in better antibody protection in comparison to vaccination with only live vaccines (Lima *et al.*, 2004). Following parenteral vaccination, by an inactivated vaccine, the immune response is mostly humoral and is highly protective (Alexander *et al.*, 2008). Types of vaccines and vaccination programs vary widely, depending on some factors (Alexander *et al.*, 2008).

To improve vaccination efficacy, vaccination programs composed of simultaneously inoculating with live and inactivated ND vaccine. In Iran ND has high prevalence and causes mortality and egg production cease and cause important economic losses, thus the prevention has notable importance. The aim of this study was to compare the antibody titers produced by Avinew live vaccines alone and in combination with inactivated vaccine administered by different routes in broiler chickens with HI and ELISA test.

Materials and Methods

Four hundred and twenty day old Ross 308 chicks were distributed randomly in 7 groups, each with 3 replicates of 20 chicks (60 chicks per group, 420 chicks totally). The ventilation, and nutrition was same in all groups. The diet was formulated by the information provided by Ross 308 management guide (Aviagen co). To evaluate the various routes of vaccination and inactivated vaccines on antibody response, Avinew vaccine (Merial Co.) used in different ways include eye drop, spray, and drinking water with or without inactivated (Merial Co.) vaccine. Vaccination was performed on days 1, 14 and 30 by different routes in various groups. Haem-agglutination inhibition (HI) and enzyme-linked immunosorbent assay (ELISA, IDEXX Laboratories) methods were used to assess antibody response at days 13, 29 and 42. For this purpose in each group 18 blood samples were taken, serums of them were separated and sent to microbiological laboratory of Tabriz Islamic Azad University. ELISA methods were done according to kit instruction. The HI test was carried out according to the standard procedure described by researchers (Kaleta and Siegmann, 1971; Majiyagbe and Hitchner, 1977).

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

Results

Results of HI antibody tests indicated there was not any significant difference between groups on day 13 of study, although at day 29 antibody titers were different between control and all other groups ($p < 0.01$). At the end of study in all groups which live vaccine was used along with inactivated vaccine antibody titers were higher than live vaccine groups. The results indicated that the spray method with inactivated vaccine has the highest antibody titers (Table 1).

Results of ELISA tests indicated at day 13 the antibody titers were highest in spray + inactivated group, it was significantly higher from eye drop and control group ($p < 0.05$). At day 29 antibody titers were different between control and all other groups ($p < 0.01$). At the end of study in all groups which live vaccine was used along with inactivated vaccine antibody titers were higher than live vaccine groups. The results indicated that the spray method with inactivated vaccine has the highest ELISA antibody titers (Table 2).

Table1: Results of antibody titration against Newcastle disease by HI test on days 13, 29 and 42

Group	HI Results		
	Day 13	Day 29	Day 42
Eye-drop vaccination along with inact. vaccine	4.60±0.24	5.52±0.54 ^b	6.02±0.32 ^c
Eye-drop vaccination	4.40±0.24	5.10±0.32 ^b	4.68±0.28 ^b
Spray vaccination along with inactivated vaccine	5.00±0.31	5.70±0.22 ^b	6.36±0.26 ^c
Spray vaccination	4.60±0.54	5.30±0.30 ^b	4.56±0.19 ^b
Drinking water vaccination along with inact. vaccine	4.80±0.37	5.42±0.35 ^b	5.62±0.20 ^c
Drinking water vaccination	4.20±0.37	5.05±0.28 ^b	4.37±0.24 ^b
Control (placebo)	4.00±0.44	2.40±0.24 ^a	1.60±0.24 ^a
P value	0.394	0.001	0.001

Table 2: Results of antibody titration against Newcastle disease by ELISA test on days 13, 29 and 42

Group	ELISA Results		
	Day 13	Day 29	Day 42
Eye-drop vaccination along with inacti. vaccine	11919.00±566.427 ^{bcd}	14449.00±520.38 ^b	15185.00±505.54 ^c
Eye-drop vaccination	10956.60±444.81 ^{abc}	14054.00±290.50 ^b	12324.00±327.48 ^b
Spray vaccination along with inacti. vaccine	13269.40±617.25 ^d	14872.80±751.86 ^b	15384.0±545.67 ^c
Spray vaccination	12031.40±737.75 ^{bcd}	14239.80±355.53 ^b	11928.20±435.38 ^b
Drinking water vaccination along with inacti.vaccine	12572.20±748.99 ^{cd}	14361.80±901.89 ^b	14468.60±270.04 ^c
Drinking water vaccination	10641.80±403.37 ^{ab}	13994.20±939.88 ^b	11712.20±424.16 ^b
Control (placebo)	10069.40±179.40 ^a	6476±480.27 ^a	4260.00±328.92 ^a
P value	0.005	0.001	0.001

Discussion

Newcastle disease important economically in all around the world and is very significant pathogen for poultry industry. The virus infects a wide variety of avian species with different clinical manifestation. Because of ND prevalence in most countries, especially in Iran, it was necessary to implement, precise controlling program for disease prevention. Newcastle disease is endemic in some countries thus considered as one of the limiting factors in poultry industry (Lana *et al.*, 1988).

Vaccination against ND widespread in world, however, failure of vaccination or improper vaccination causes ND outbreaks widely. It was reported, generally good vaccination programs provides relative protection against disease and mortality, but virus transmission and epidemics of ND could be continue in vaccinated flocks. Previous studies was reported for prevent from spreads of disease in vaccinated birds it should be that more than 85% of them has high antibody titers (log₂ titer ≥3) after vaccination (van Boven *et al.*, 2008). Researcher indicated it is possible to obtain high antibody titers in vaccinated birds, but the vaccine content and administration techniques are very important in outcomes of vaccination programs (van Boven *et al.*, 2008). Studies showed that there is no significant different protection against various viruses after Avinew vaccination, however the protection against ND challenge is dose dependent and in farm condition 10^{6.0} EID₅₀ could protect chickens from mortality. Protections obtains from vaccination could not prevent replication of virus and infection, and in some cases gross lesions were seen in birds (Bwala *et al.*, 2009). Also researchers indicated that the vaccination could protect birds from virus replication in host tissues, although they were reported virulent strains infect and replicate in vaccinate birds (Miller *et al.*, 2007). In vaccinate flocks infection, shedding, and transmission of virulent ND viruses almost are only with less clinical signs and mortality (van Boven *et al.*, 2008). Therefore, if preventive vaccination programs are suitable, sufficient immunity levels will achieved in chicken flocks to prevent economic losses (Capua and Marangon, 2006).

In a research that was compare La Sota vaccine intraocular and Mukteswar vaccine by the drinking water route, the results demonstrated that the La Sota vaccine has highest titer of HI antibodies and Mukteswar has lowest titers of HI antibody against ND prior to challenge (Rehmani, 1996). It was reported that the immune response following vaccination by drinking water route dependent on the strain of ND vaccine. F strain and La Sota vaccines produce 85.90% protection against challenge, whereas the Mukteswar strain only provide 45% protection. Drinking water containing the vaccine should be drunk as soon as possible and the environmental conditions and age of vaccination affects the outcome of serologic response (Rehmani, 1996).

In Iran for controlling ND, different live and inactivated vaccines are available, but ND continuously causes losses in poultry populations, therefore it is necessary to indicate the serological response to vaccination and different routes of vaccination in birds. Studies reported serologic response to primary vaccination were similar but after second vaccination has been shown that the La Sota and V4 vaccines more effective than RDFV vaccine (Roy *et al.*, 1998).

Inactivated NDV vaccines could protect birds from morbidity and mortality, but for decreases of replication of virus, more specific levels of antibodies comes from same genotype as the challenge viruses were

required. However, it was shown that the heterologous antibodies prevent virus transmission. Higher level of antibodies in vaccinated birds, decrease infected birds and virus shed (Miller *et al.*, 2013).

Evaluation of various commercial ND live and inactivated vaccines by various vaccination routes indicated that the various live ND vaccines has produce similar antibody responses but inactivated ND vaccines produce different levels of protections (Lin *et al.*, 1990). Vaccination against ND by spray and eye drop method showed that in spray method HI antibody titers was higher than eye drop method (Landman *et al.*, 2017). It was indicated the most effective route for vaccination against ND was aerosol, which is followed by the intraocular method, and intra-tracheal administration or subcutaneous inoculation led to a marginal response. The aerosol route of vaccination produce the highest levels of antibody, despite the lesser amount of vaccinal virus (Beard and Easterday, 1967).

Our results demonstrated that there was significant difference from the aspect of the antibody titers produced by the live + inactivated vaccine, thus inactivated vaccine administration to broiler chicken were recommended. In addition, our results indicated that the antibody response in the spray routes of vaccination along with inactivated vaccination was highest in both HI and ELISA. Because of vaccination, reaction in spray method it seems eye drop vaccination along with inactivated vaccine could be effective and if there was not any predisposing factor in chickens such as a bacterial infections, spray method could be used with success.

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