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Research Article

Preparation and Competative Immune Responce Evaluation of Infectious Bronchitis (H-120) + Newcastle Disease (La-Sota) Live Bivalent Vaccine

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Abstract: *Newcastle* disease (ND) and *Infectious Bronchitis* (IB) are highly contagious, acute and common poultry viral diseases. Control of these two important diseases of poultry industry was based on biosecurity and vaccination program. There is discussion about viral interference between these two viruses when combined. The aim of this research was to assess the effectiveness of a Razi live bivalent vaccine containing *LaSota* strain of *Newcastle Disease Virus (NDV)* and *H-120* strain of *Infectious Bronchitis V*irus *(IBV)*. The bivalent vaccine was formulated based on EID_{50} titer of viruses. The immunogenicity of the vaccine was compared with commercial and monovalent Razi live *IB (H-120) and ND (La Sota)* vaccines in Specific pathogen free (SPF) and commercial chickens. The vaccination response was evaluated by haemagglutination inhibition (HI), serum neutralization and enzyme linked immunosorbent assay (ELISA) antibody titers. SPF chickens that had received one dose of the Razi bivalent vaccine, has antibody titer (HI) of *Newcastle* 5.87 based on Log2 and the serum neutralization index of Infectious bronchitis 6.5. Geometric mean antibody titers (GMTs) of HI were 3.20 and 3.30 for Razi bivalent vaccine and commercial one respectively. Serum *IBV* ELISA antibodies GMTs were 3569 and 1992 and 320 for Razi bivalent vaccine and commercial one and control group respectively. Therefore antibody titers against *NDV* and *IBV* in chickens that received Razi vaccine were similar to those that were given monovalent and commercial vaccine. Our results show that the combined ND+IB vaccine has the ability to induce a high level of immune response in vaccinated chickens and no interference was seen between Razi and commercial one.

Keywords: Interference, Poultry, Virus, Immunization, Combined Vaccine,

INTRODUCTION

Newcastle disease (ND) and *Infectious Bronchitis (IB)* are two important viral diseases that can cause significant economic losses to the poultry industry (Cavanagh, and Naqi, 2003; Alexander D.J. 2003). *Newcastle Disease Virus (NDV)* is belongs to *Paramyxoviridae* and is one of the most important viral diseases of poultry industry of most countries by causing avian *Pneumoencephalitis* and other fatal diseases (Alexander D.J. 2003; OIE, 2012). *Infectious bronchitis (IB)* is an acute and highly contagious viral disease of chicken which appears with severe epidemic of all ages and high mortality in chickens less than 6 weeks *Infectious Bronchitis Virus (IBV)* is a *gammacoronavirus* of the family *Coronaviridea* (Cook, 2001; Cavanagh, & Naqi, 2003). Control of these two important viral diseases of poultry involved biosecurity measures and vaccination. To reduce production costs, simultaneous immunization with two or three vaccines, has become a common practice in poultry industry; like bivalent *Newcastle* and *Infectious bronchitis* vaccine (Cardoso et al., 2005; Bande et al. 2015). Meanwhile, the uses of live vaccines due to high economic costs have encouraged the poultry farm owners to use bivalent or polyvalent live or inactivated vaccines due to use of such vaccines is so affordable. In addition to the economic aspects, the use of bivalent vaccines could reduce the

mortality of flock and other harmful side effects following repeated vaccination. Because of the importance of *NDV* and *IBV* several administration methods have been introduced for design and production of bivalent vaccines (Markham et al., 1956; Bengelsdorff, 1972; Zygraich et al., 1973; Winterfield ,1984;). Interference between *IBV* and *NDV* has evaluated by some researcher (Raggi & Lee, 1964; Bracewell et al., 1972; Thornton, Muskett et al., 1975 ;) as between *Influenza* virus and *Newcastle* disease virus (Ge et al. 2012,). Currently effect of viral interference on replication of viruses evaluate by RT-PCR; (Gelb et al. 2004; Ge et al. 2012).The objective of this study was to study effectiveness of the developed combined vaccine under experimental conditions and evaluate the effect of vaccine on the immune response against ND and IB in broilers.

MATERIAL AND METHOD

preparation of live bivalent vaccine

propagation of viruses: The *H-120* strain of *IBV* and *La Sota* strain of *NDV* were propagated separately by inoculating 9-to-11-day-old embryonated chicken eggs (Venky's Company, India) via the allantoic cavity according to standard (European pharmacopoeia, 2005; OIE, 2010). After incubation of inoculated eggs at 37° C, the allantoic fluid of the inoculated eggs was harvested in sterile condition.

virus titration: The titer of two viruses was carried out with inoculating 9 -11 day old embryonated SPF eggs with serial dilution of harvested *IB* and *ND* viruses according standard. The viral titers were calculated by Spearman-karber method (Pedro Villegas, 2008; OIE, 2012).

***vaccine formulation*:** The formulation and final mixing of two viruses was done based on titers of two viruses and the stabilizer solution was added to the final formulated bulk. Freeze drying of the vaccine was done and quality control test of bivalent vaccine was carried out according to OIE (2012).

safety: Safety of bivalent vaccine was carried out according to section C.4.b of Chapter 2.3.2 of OIE (2012). The vaccine was inoculated to ten two-week susceptible SPF chickens (ten doses per bird) by eye drop rout, and the vaccinated chickens were examined daily for a period of 21 days in terms of local and general reactions. The vaccine will be approved regarding health if no abnormal response or symptoms will occur in the chickens (European pharmacopoeia, 2005; OIE, 2012).

serologic tests

serum neutralization (SN): SN test was carried out in 10 days old embryonated SPF eggs. All sera were inactivated at 56° C for 30 minuets. Ten fold dilutions of virus were prepared, and each dilution was mixed with equal volume of antiserum. The virus serum mixtures were incubated at room temperature for 30 minutes before inoculation. Each mixture was inoculated into five eggs. Titer of virus alone and virus + serum mixture was calculated by Kerber method. Neutralization index was calculated by deducting the serum + virus mixture titer from the pure virus titer (European pharmacopoeia, 2005; Thayer & Beard, 2008; OIE, 2012).

hemagglutination inhibition (Hi): HI test was performed according to OIE guidelines and Alexander et al. (1983). Serial dilutions were prepared from separated sera based on Log_2. Then, 4 units of Newcastle antigen were added to the serum dilutions. After the incubation time of 30 minute, washed 1% red blood cells were added in each well. The antibody titers were determined according to the latest dilution of serum that inhibited the hemagglutination (European pharmacopoeia, 2005; Thayer & Beard, 2008; OIE, 2012).

enzyme linked immunosorbent assay (ELISA): It was carried out for detection of antibodies against NDV and IBV of bivalent vaccines according to kit manufacture (Biochek Co.).

experimental design

potency tests of the vaccines in SPF chicks

Ten SPF chickens at 21 days of age in two separate groups were tested. The first group, including 10 chickens, was vaccinated with one dose of Razi bivalent vaccine by eye drop rout. The second group was kept as controls in a separate location. Four weeks after

immunization of chickens, blood samples were taken from the vein under the wing of all the chickens to determine antibody titers for *NDV* by HI and for *IBV* by serum neutralization method to determine serum index, and the sera were stored at - 20 ° C until use (European pharmacopoeia, 2005; OIE, 2012).

potency tests of the vaccines in commercial chickens

Potency evaluation of Razi bivalent vaccine was carried out in commercial broiler chickens in two experiments. 480 one-days-old commercial broiler chickens were divided into 4 experimental groups. Vaccination program was shown in table 1 and 2. Blood sample were collected at intervals of ten days to 50 and at 35, 45 and 55 days post vaccination in experiment 1 and 2 respectively. The sera were stored at -20 ° C until analysis.

RESULTS

The antibody response of Experimental and commercial bivalent vaccine was determined by HI, SN and ELISA test. Table 3 and 4 show that group received Razi vaccine gave NI and ELISA titer alternates between 3.8, 4.4, 4.20 , 5.8 and 2772, 2914, 3019 and 3569 in days 20, 30, 40 and 50 days of age respectively (Figure 1 & 2). Regarding to group received Razi bivalent vaccine, the ELISA antibody titers increased gradually from 20 days (3.8 and 2772) till record the highest level in 50 days (5.8 and 3569). Group 2 which received commercial bivalent vaccine, the both antibody titers (SN and ELISA) were 2.6, 4.2, 5.2, 6.2 (SN titer) and 1833, 1514, 1534, 1992 (ELISA titer) post vaccination respectively that no significant differences were seen. Table 5 show the mean HI titers of ND in the vaccines used in this study at (20 – 50) days post vaccination were (3.10, 3.40, 4.00, and 3.20) for Experimental vaccine and (3.40, 4.10, 4.0, 3.30) for commercial one. In this case no important differences were observed (Figure 3).

In group which vaccinated with monovalent vaccine (table 6 and 7), the NI value (IB) were 5, 4.15, 6.5 and mean HI titer of (ND) were 4.4, 2.20, 3.4 at 35, 45 and 55 days post vaccination while these were 5, 5.2 , 5.8 (IB) and 4.4, 2.26 , 2.92 (ND) for Razi bivalent vaccine (Figure 4 and 5).

The mean HI antibody titer in SPF chickens that had received one dose of bivalent vaccine was equal to 5.87 four weeks after inoculation compared with the control group with a mean of about 2.2 based on Log_2. Their serum index was equal to 5.6 while it was as ≤ 1.5 in the control group. HI antibody titers of control groups against NDV in two experiments, decreased from GMT 2.3 in the first day to 1.0 at 50 days post vaccination. ELISA antibody titers against IBV decreased from GMT 2156 to 625.

DISCUSSION

Newcastle disease and Infectious Bronchitis are highly contagious and economically important viral diseases in poultry industry causing significant losses (Cavanagh & Naqi, 2003; Alexander. 2003). Vaccination is the most cost approach to control these two diseases. Effectiveness and safety of ND and IB vaccines have been demonstrated. lentogenic strains of NDV and attenuated strains of IBV are widely used and effective in preventing disease and cause successful immunity. These vaccines are made commercially available (Senne et al. 2004; Bande et al. 2015). For high economic cost and logistics using several live vaccines has encouraged the poultry farm owners to use bivalent or polyvalent vaccines. This type of vaccines from the economic aspect, decreasing the stress caused by repeated inoculations, less mortality in the flock, time and eliminating other harmful agents have come to the focus of attention and use of these type of vaccines in poultry industry is increased. Since these vaccines are used once instead of multiple times of inoculations, which minimizes the indirect complications and losses of stress and the presences of vaccinators. Thus, the owners of large herds of poultry are interested in this type of vaccines.

If inoculation is done separately for each individual vaccine, there is a possibility that each of them will fail to induce its appropriate level of protection in vaccinated birds. (Winterfield, 1984; Cardoso et al., 2005).

Cavanagh and Naqi (2003) have reported that if IB virus titer was more than ND virus interference may occur hence combined vaccine are preferred to mixing monovalent vaccine (Cook, 2001; Ge et al. 2012).Thus, mixing two single ND and IB vaccines and inoculation of its regardless of viral titers could cause interference between these two viruses and fails to induce an effective immunity, and therefore, no proper protection will be seen against the challenge of acute virus (Raggi & Lee, 1964; Bracewell et al., 1972; Thornton & Muskett et al., 1975; Winterfield, 1984). Optimization of ND and IB viruses in the bivalent vaccine is the most important factor in obtaining an optimal protective immunity (Winterfield, 1984). In making the combined vaccine, the formulation of vaccine and the titer of viruses having viral interference with each other under normal conditions are of vital importance (Markham, 1956; Winterfield, 1984; Ge et al. 2012). Our results in this research are in accordance with other same research (Cardoso et al. 2005; Zamani Moghaddam et al. , 2007).

In evaluation of combined vaccine in commercial broiler chickens, Razi bivalent vaccine with a commercial one was used for immunization of two groups of commercial chickens, and the vaccinated chickens were evaluated until about 50 days post vaccination. HI antibody titer of NDV and serum index of IBV in two groups that vaccinated with Razi vaccine and commercial vaccine at 35 days after inoculation were respectively as 3.20 and 3.30 based on Log_2, and 6.5 and 5.8. ELISA antibody titer of IB in these two groups was 3569 for Razi vaccine and 1992 for commercial one. In the second experiment, no adverse reaction was observed in pullets during about two-month period of study. In the control group, decrease in NDV antibodies titers and negative antibody of IBV in the controls indicated the absence of acute infection with the virus during the trial period. In our study it was observed that interference did not occur in the group that was given the IBV+NDV combined vaccine in compare with groups that were given monovalent of IB and ND or commercial vaccines according to Zygraich et al. (1973) and Winterfield, (1984) which have reported there is no interference between IBV and NDV.

It was concluded that mixing two single ND and IB vaccines, without considering viral titers can cause interference between two viruses and would not create an effective immunity, and thereby, no proper protection will be seen against challenging with an acute virus. Thus, optimization of NDV and IBV in the combined vaccine is very important factor in obtaining an optimal protective immunity and administrating combined ND +IB vaccine which is manufactured preferred to using separate administration of these two vaccines.

Ethics. I hereby declare all ethical standards have been respected in preparation of the submitted article.

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Table 1: vaccination program (experiment 1)

Groups	No. of birds	Age of Vaccination	Type of vaccines
1	100	10 days	Razi bivalent vaccine
2	100	10 days	Commercial bivalent vaccine
3	40	-	Control group (non-vaccinated)

Table 2: vaccination program (experiment 2)

Groups	No. of birds	Age of Vaccination	Type of vaccines
1	100	18	La-Sota vaccine
		21	H-120 vaccine
2	100	21	Razi bivalent vaccine
3	40	-	Control group (non-vaccinated)

Table 3: The IB NI response to tow type bivalent IB + ND vaccines (experiment 1)

Groups	Type of vaccines	Mean antibody titer				
		Days post vaccination				
		10	20	30	40	50
1	Razi bivalent	5.4	3.8	4.4	4.20	5.8

	vaccine					
2	Commercial bivalent vaccine	5.4	2.6	4.2	5.2	6.2
3	Control group (non-vaccinated)	5.4	2.0	1.6	1.5	1.9

Table 4: The IB ELISA antibody response to tow type bivalent IB + ND vaccines (experiment 1)

Groups	Type of vaccines	Mean antibody titer				
		Days post vaccination				
		10	20	30	40	50
1	Razi bivalent vaccine	2741	2772	2914	3019	3569
2	Commercial bivalent vaccine	2146	1833	1514	1534	1992
3	Control group (non-vaccinated)	2146	792	635	750	625

Table 5: The ND HI antibody titer to two type bivalent IB + ND vaccines (experiment 1)

Groups	Type of vaccines	Mean antibody titer				
		Days post vaccination				
		10	20	30	40	50
1	Razi bivalent vaccine	2.30	3.10	3.40	4.00	3.20
2	Commercial bivalent vaccine	2.30	3.40	4.10	4.00	3.30
3	Control group (non-vaccinated)	2.30	1.3	1	1.0	1.0

Table 6: The IB NI antibody titer to bivalent IB + ND and monovalent vaccines (experiment 2)

Groups	Type of vaccines	Mean antibody titer		
		Days post vaccination		
		35	45	55
1	Razi monovalent vaccine	5.0	4.15	6.5
2	Razi bivalent vaccine	5.0	5.2	5.8
3	Control group (non-vaccinated)	1.9	1.7	2

Table 7: The ND HI antibody titer to bivalent IB + ND and monovalent vaccines (experiment 2)

Groups	Type of vaccines	Mean antibody titer		
		Days post vaccination		
		35	45	55
1	Razi monovalent vaccine	4.4	2.2	3.4
2	Razi bivalent vaccine	4.4	2.26	2.92
3	Control group (non-vaccinated)	1.0	1.0	1.2

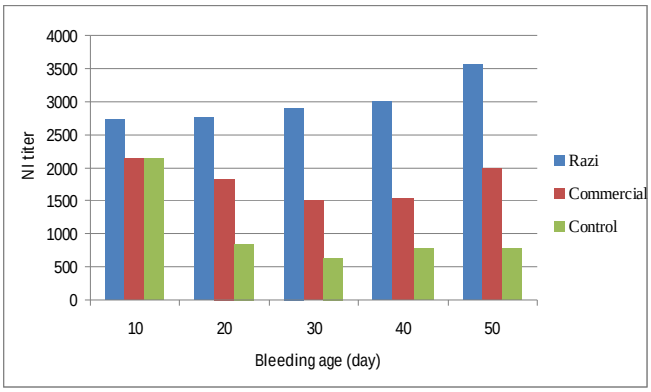

Figure 1: Antibody titers against Infectious bronchitis virus of groups 1, 2 and control in first experiment

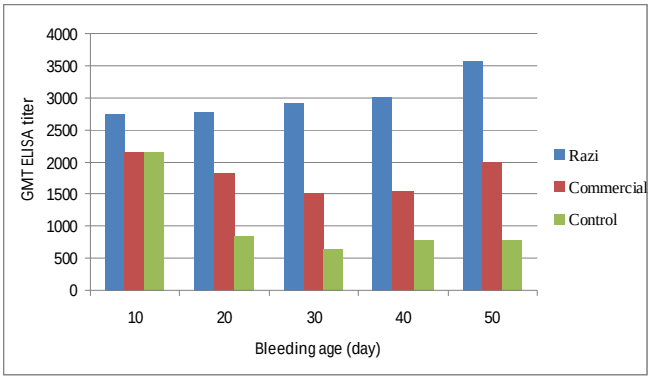

Figure 2: ELISA titers against Infectious bronchitis virus of groups 1, 2 and control in first experiment

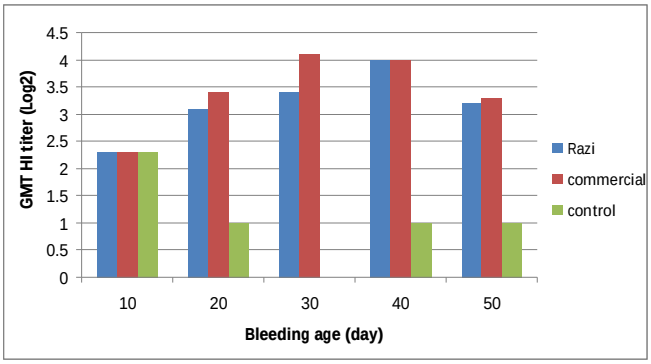

Figure 3: HI titers against Newcastle disease virus of groups 1, 2 and control in first experiment

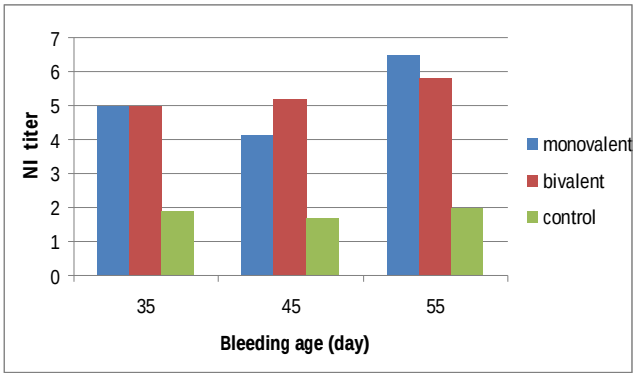

Figure 4: NI titers against Infectious bronchitis virus of groups 1, 2 and control in second experiment

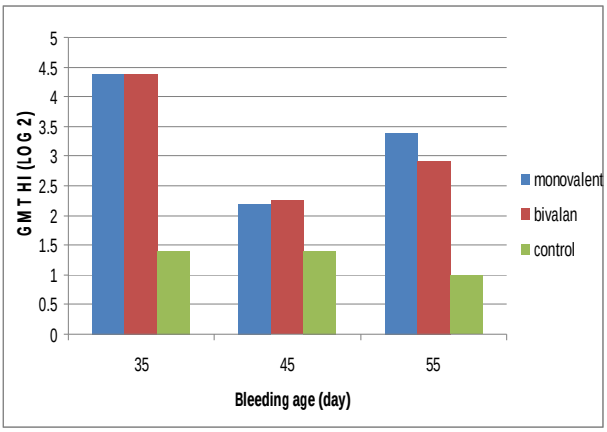

Figure 5: HI titers against Newcastle disease virus of groups 1, 2 and control in second experiment

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