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The effect of mixed live vaccines of Newcastle disease and infectious bronchitis on systemic and local antibody responses in chickens

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Summary

In the present study, 360 male day-old broiler chicks were used to determine the effect of mixed live vaccines of Newcastle disease (ND) and infectious bronchitis (IB) on serum and local antibody responses to IB. Chicks were randomly divided into 12 treatment groups of 3 replicates and reared for 40 days on floor pens. Groups 1 to 5 received mixed ND and IB vaccine. Groups 7 to 11 received IB vaccine alone and regarded as positive controls. Group 6, that received ND vaccine only, and group 12, that received no vaccine, were considered as negative controls. Antibody titer against IB in the nasal washings and sera was measured using enzyme-linked immunosorbent assay (ELISA). The chickens were inoculated with IB live vaccine (strain H120) with or without ND live vaccine by means of an eye dropper. Serum samples obtained on the 19th and 29th day of age and serum samples, as well as nasal washings, collected on day 40, were used to determine systemic and local antibody responses to IB. On the 40th day of age, tracheal samples were also collected to study the probable pathologic lesions due to the effect of live vaccines used. There was no significant difference in systemic antibody response (SAR) among all groups measured on the 19th day. On the 29th and 40th day of age, SAR of the negative control groups was significantly ($P<0.05$) different from those received IB vaccine. On the 40th day, nasal washings showed higher antibody titer as compared to the systemic antibody titer ($P<0.05$). The pathologic lesions in groups received mixed vaccine were higher than groups received IB vaccine alone or the control groups. Mortality rate, weight gain and food conversion ratio were not significantly different among groups studied. Despite the observations that mixed live IB and ND vaccination resulted in a higher antibody response as compared to the single IB vaccination, yet this program could not be recommended to use in farm due to higher pathologic lesions in trachea.

Key words: Infectious bronchitis, Newcastle disease, Mixed vaccine

Introduction

Infectious bronchitis (IB), as a very contagious respiratory disease of chickens, was firstly described in North Dakota, USA, in 1930 (Cavanagh and Naqi, 2003). Nowadays, it has been spread all over the world (Cook, 2001; Cavanagh and Naqi, 2003). Prevention of the disease by immunization is worthwhile due to contagious nature of the disease and occurrence of numerous serotypes of IB virus (IBV) (Cavanagh and Naqi, 2003). Nevertheless, prevention of the disease by vaccination has been associated with partial success.

Histopathologic studies of IBV have well addressed (Kotani *et al*., 2000).

Newcastle disease (ND), for the first time was described in Java, Indonesia. In 1927, isolation of ND virus (NDV) was reported in England. There was another report from Betava, Indonesia.

Very limited studies have addressed the effect of NDV on IB vaccination. In a comparative study, Thornton and Muskett,

(1973), examined immunization of ND vaccine alone or in combination with IB vaccine. The results of this study indicated that in those chickens received B1 vaccine alone, the mean haemagglutination inhibition (HI) titer against ND was higher than those received mixed vaccine (B1 and H120). The difference, however, was not significant. CID_{50} of the challenge virus for the group received B1 vaccine alone was significantly higher than the group received mixed B1 and H120 ($10^{10.13}$ vs $10^{9.01}$) (Thornton and Muskett, 1973). Results of this study indicated that protection of chickens against challenge of virulent NDV is higher in those chickens received B1 alone, as compared to those received B1 and H120. The tolerable ELD_{50} for the chicks received ND vaccine and the chicks received ND in combination with IB vaccine were $10^{9.65}$ and $10^{7.98}$, respectively ($P<0.05$) (Thornton and Muskett, 1973).

In the current study, the effects of ND vaccine on humoral or local immunity responses to IB vaccine were studied.

Materials and Methods

Three-hundred and sixty Ross (208) day-old male broiler chicks were divided into 12 groups of 30 chicks (3 replicates of 10 in each group), and were placed on litter pens. Groups 1–5 received mixed ND and IB vaccine (Table 1). Groups 7–11 received IB vaccine alone and considered as positive control (Table 2). Each of the treatment groups had a corresponding positive control (1 vs 8; 2 vs 9; 3 vs 10; 5 vs 11).

Groups 6 and 12 were considered as negative controls; the former received ND vaccine alone and the latter received no vaccine.

Feeding regimens of all groups were adjusted as recommended by NRC (1994). Vaccination was performed according to the program, and serum samples were taken on the 19th, 29th and 40th day of age to be tested for IB ELISA at 405 nm (KPL Inc., Synbiotic Co., USA). Nasal washings were prepared from head samples on the 40th day of age and used for measuring IgA against IB determined by ELISA. The mean body weight and food conversion ratio (FCR) on the 39th day and FCR during the first to 39th day of age were measured. In addition, histopathologic lesions scoring on the 40th day of age was determined (Nakamura *et al*., 1992). Scoring was performed according to the lesions: score 0 shows no lesions; score 1 shows hyperemia and infiltration of inflammatory cells; score 2 shows hyperemia, infiltration of inflammatory

Table 1: Characteristics of groups 1–5, for determination of the effect of IB and ND simultaneous vaccination and negative control (group 6)

Treat	B1 when 8-day-old	B1 + IB when 8-day-old	Lasota1 when 21-day-old	Lasota1 + IB when 21-day-old	Lasota2 when 31-day-old	Lasota2 + IB when 31-day-old
1		*	*		*	
2		*		*	*	
3		*		*		*
4	*			*	*	
5	*			*		*
6	*		*		*	

*Vaccine received

Table 2: Positive control groups (7–11) received IB vaccine (H120) at different ages and negative control (group 12)

Treat	IB when 1-day-old	IB when 8-day-old	IB when 21-day-old	IB when 31-day-old
7	*			
8		*		
9		*	*	
10		*	*	*
11			*	*
12	No vaccine received			

*Vaccine received

cells, edema and deciliation; and score 3 shows hemorrhage, desquamation and hyperplasia. Data obtained from the experiment were analysed in a complete randomized block design using general linear model (GLM) procedure of SAS (statistical analysis system, SAS Inc., 1995). In case of significant difference Tukey's test was used.

Results

On the first day of age, the mean ± SD level of antibodies against IB was 2418 ± 315 determined by ELISA. ELISA results from IB serological examinations on the 19th, 29th and 40th day of age and from nasal washings (IgA) on the 40th day of age, are summarized in Table 3. Table 4 summarizes the results related to the performance criteria on the 39th day of age. Histopathologic lesions scoring on the 40th day of age are summarized in Table 5.

Discussion

On the 19th and 29th day of age, there was no significant difference in terms of antibody titers between the groups received mixed vaccine and those received single vaccine. Groups 6 and 12 (negative controls) and the groups which did not receive IB vaccine up to the 19th day of age, had still remarkable antibody titers, perhaps, because of remaining maternal antibody. There are a number of reports in agreement with these results (Thornton and Muskett, 1973; Cook, 2001). These antibody titers decreased to zero, thereafter. On the 29th day of age, antibody titers of the treatment groups received mixed vaccine were lower than those received IB vaccine alone, but these differences were not significant.

On the 40th day of age, results indicated

Table 3: IB, serum ELISA titers (log2) and IB, ELISA titers related to IgA levels in nasal washings (log2)

Treatments	Mean±SD titer on 19th day (Serum Ab)	Mean±SD titer on 29th day (Serum Ab)	Mean±SD titer on 40th day (Serum Ab)	Mean±SD IgA titer in nasal washings
1	5 ± 1.22	4.9 ± 1.4^{c}	6.1 ± 1.01^{d}	9.03 ± 1.04^{ce}
2	4.9 ± 0.89	6.1 ± 1.04^{ab}	9.8 ± 1.5^{ac}	10.11 ± 0.58^{bd}
3	4.7 ± 1.5	6.3 ± 1.1^{a}	10.5 ± 1.7^{a}	12.62 ± 0.43^{a}
4	4.3 ± 1.1	5.3 ± 0.95^{c}	6.9 ± 1.3^{d}	10.07 ± 0.81^{be}
5	3.8 ± 1.5	5 ± 1^{c}	9.2 ± 1.4^{bc}	11.06 ± 0.33^{b}
6	3.5 ± 0.75	0^{d}	0^{e}	1.52 ± 1.44^{g}
7	4.9 ± 1.05	5.7 ± 1.1^{bc}	6.8 ± 1.4^{d}	7.06 ± 0.74^{f}
8	4.7 ± 1.11	5.8 ± 1.15^{bc}	6.6 ± 1.2^{d}	7.18 ± 0.89^{f}
9	3.9 ± 1.4	6.7 ± 1.1^{a}	7.05 ± 1.05^{d}	9.15 ± 0.54^{d}
10	4.2 ± 0.8	6.8 ± 1.5^{a}	8.9 ± 1.7^{b}	10.98 ± 0.1^{b}
11	3.9 ± 1.4	5.9 ± 1.8^{bc}	8.4 ± 1.6^{b}	9.27 ± 0.7^{de}
12	3.1 ± 0.95	0^{d}	0^{e}	1.42 ± 1.44^{g}

Table 4: Mean±SD body weight and FCR in 39-day-old chickens

Treatment	Mean body weight (gr)	FCR
1	1725 ± 38.68	1.81 ± 0.03
2	1720 ± 39.17	1.90 ± 0.09
3	1683 ± 27.10	1.82 ± 0.03
4	1740 ± 45.56	1.83 ± 0.04
5	1710 ± 32.66	1.80 ± 0.09
6	1773 ± 32.68	1.77 ± 0.04
7	1766 ± 38.31	1.88 ± 0.07
8	1760 ±24.72	1.83 ± 0.04
9	1730 ± 22.78	1.89 ± 0.07
10	1690 ± 31.69	1.90 ± 0.11
11	1761 ± 103.27	1.83 ± 0.08
12	1810 ± 34.57	1.80 ± 0.08
NS	NS	NS

NS = non significant

Table 5: Mean±SD histopathologic lesion scores evaluated on the 40th day of age

GT	1	2	3	4	5	6	7	8	9	10	11	12
LS	0.93 ±0.25[a]	1.2 ±0.24[a]	1.46 ±0.26[a]	1.06 ±0.21[a]	1.2 ±0.24[a]	0.2 ±0.11[b]	0.33 ±0.16[b]	0.33 ±0.16[b]	0.46 ±0.17[b]	0.46 ±0.19[b]	0.46 ±0.19[b]	0.2 ±0.11[b]

LS: Lesion score: score 0 shows no lesions; score 1 shows hyperemia and infiltration of inflammatory cells; score 2 shows hyperemia, infiltration of inflammatory cells, edema and deciliation; score 3 shows hemorrhage, desquamation and hyperplasia. GT: Treatment groups

that groups with more frequent IB vaccination had higher antibody titer so that group 3 (3 times mixed vaccination) had the maximum antibody titer ($P<0.05$) (Table 3). At the same age, groups received mixed vaccine had numerically higher antibody titers as compared to the groups received IB vaccine alone. In this regard, the results show significant differences between groups 2 and 9 and also between groups 3 and 10 ($P<0.05$). This might be due to the increased histopathologic lesions in the trachea of the treatment groups, which may assist to increased immune response. Administration of mixed vaccine has demonstrated to induce deciliation, hyperplasia, hyperemia and some lesions in the tracheal epithelial mucosa (Nakamura *et al*., 1992). There is a considerable variation in response of birds to mixed vaccines. Smith (2003) reported that this variation was associated to the IBV strain. The only exception in this case was group 1 receiving mixed vaccine including ND + B1, which showed lower titer than its positive control (group 8), though the difference was not statistically significant.

In general (with some exception), trend changes in IgA titers were similar to that observed in systemic antibody titers.

Cholakova (1985) studied the immune response induced by live mixed ND (Lasota) and IB (H120 or H52) vaccines. The results showed that simultaneous use of these three vaccines had not any positive or negative effects on the immune response as compared to the administration of a single or combined vaccines.

Cavanagh and Naqi (2003) have declared that excess IBV particles in the vaccine may interfere with NDV immune response. By virtue of this, it is suggested that combined vaccines are preferred to mixed single vaccines (Cook, 2001). As a general rule, it is indicated that two distinct live vaccines should not be mixed for use and it ought to be administered separately with 14 (at least 7) days interval to avoid any possible interference (Cook, 2001).

Monitoring the level of serum antibodies is commonly used as an index to assess the amount of protection induced by vaccination against IBV (Cook *et al*., 1991). In IBV infections, the level of serum antibodies plays a very important role in prevailing the infection. However, a direct relationship between the serum antibody titer and the level of protection against the infection was still not shown. In fairness to this, the chickens with very low level of serum antibodies were protected against the pathogenic virus (Chabra and Peters, 1985; Cook *et al*., 1991). It has been recently shown that mucosal immunity in the respiratory system acts as the first line of defense against IBV challenge and resistance against IBV infections may be due to either tracheal mucosal immunity (Chabra and Peters, 1985) or cell mediated immunity (Pakpinyo and Sasipreeyajan, 1993). There are some reports indicating a significant relationship between mucosal (local) IgA and resistance against infection with IBV. Therefore, measuring local IgA could be a suitable alternative for monitoring the level of protection after IBV vaccination or infection (Beard, 1968; Sharma and Adlakha, 1994).

Despite the fact that there is not any report regarding the effect of mixed ND + IB vaccine on IB antibody titers, the result of this study indicated that mixed ND + IB vaccine induced higher systemic and local antibody responses as compared to the administration of IB vaccine alone. However, because of histopathologic lesions induced, this method may not be recommended.

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