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Original Article

Effect of the Commercial Mixed Live Newcastle Disease and Infectious Bronchitis Vaccines and the Use of Two Separate Vaccines Given Simultaneously on Systemic Antibody Responses in Chickens

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Abstract

Background and Aims: The objective of this study was to investigate the effects of Newcastle Disease and Infectious Bronchitis vaccination programs in chickens.

Materials and Methods and Results: In the present study, 225 day-old broiler chicks divided into 5 groups. The groups were submitted to vaccinations, except for the non-vaccinated control group. The chickens in the groups 1 were kept as a control group and did not receive vaccine. The chickens in the group 2 were vaccinated with commercial mixed vaccine. The chickens in the groups 3 were vaccinated with two separate vaccines simultaneously. The chickens in the group 4 were vaccinated with single ND vaccine. The chickens in the groups 5 were vaccinated with single IB vaccine. Sera were collected at 18 and 45 days of age and submitted to serologic tests to assess antibody levels.

Conclusion: Results demonstrate that there is not any significant statistical difference between the vaccinated groups.

Keywords: Immune response; Newcastle Disease; Infectious Bronchitis; Commercial mixed live vaccines (IBV+NDV); Broiler chicks

Introduction

Newcastle Disease (ND) and Infectious Bronchitis (IB) are important diseases in the poultry industry and cause great losses (King and Cavanagh, 1991, Tu *et al*, 1998). Control involves the use of biosecurity procedures and vaccination. In order to reduce costs, vaccination using two or three vaccines simultaneously became a common practice in poultry production, such as a combined vaccine against ND and IB. Infectious Bronchitis virus and Newcastle Disease virus are prevalent in all countries with an intensive poultry industry. Vaccination is only partially successful due to the continual emergence of antigenic variants.

Newcastle disease vaccins

Basically, there are three types of commercially available vaccine for Newcastle Disease: live lentogenic, live mesogenic and inactivated (Alexander, 2003). Typical vaccine strains are Hitchner B1 and La Sota – possibly the two most widely used animal vaccines – and F strain and V4. However, these viruses have frequently been subjected to selection pressures by manufacturers in order to improve their immunogenicity or to enable their use by a particular method of application

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(Alexander, 2003). Live lentogenic vaccines may be given to birds individually by eye drop or beak dipping but it is usually more practical to use methods of mass application such as in the drinking water or by machines generating sprays or aerosols. Use of aerosols of La Sota vaccine on such birds may result in heavy mortality (Alexander, 2003).

Infectious Bronchitis vaccins

Live attenuated and inactivated (oil-adjuvanted) vaccines are highly effective and widely used. The live attenuated vaccines are used to prevent and control infection in young birds and to 'prime' future breeders and layers prior to administration of inactivated vaccines (Cavanagh and Naqi, 2003). It is important to remember that, for inactivated vaccines to be effective, chickens must have been 'primed' with a live vaccine.. (Cavanagh and Naqi, 2003). Despite the apparent protective effect of maternally derived immunity in very young chicks, live vaccines can be administered successfully from 1 day of age by coarse spray, beak dipping or nasal or eye drop. Older birds may be vaccinated via the drinking water, by eye drop or coarse spray. Different vaccination protocols are available, designed for different types of bird (Cavanagh and Naqi, 2003) but the most important point is to ensure that the vaccine is carefully and correctly administered so that each bird receives the required dose. For broilers this is likely to be given in the hatchery by coarse spray. Revaccination of broilers, possibly at 2–3 weeks of age, is now common practice in some areas (Cavanagh and Naqi, 2003).

Interference between Newcastle Disease Virus and Infectious Bronchitis Virus

In order that protection against both Infectious Bronchitis and Newcastle Disease may be achieved by one application of vaccine, the two vaccines may be combined. Since the risk that, if it is present in excess, the Infectious Bronchitis vaccine may interfere with the response to the Newcastle Disease vaccine, the use of a combined product is preferable to the use of two separate vaccines given together (Cook, 2008). Earlier studies have reported that Infectious Bronchitis virus (IBV) interferes with the immune response against Newcastle Disease virus (NDV) (Bracewell *et al*,1972; Raggi and Lee,1964; Thornton and Muskett, 1975). Smith (2002) and other authors report productivity losses related to viral interference in broiler flocks in the southeast region of the United States, resulting in economic losses to producers. Thus, the objective of this work was to evaluate the effect of associated vaccines on the immune response against Newcastle Disease and Infectious Bronchitis in broilers (Smith, 2002).

Methods

Chickens

A total of 225 day-old broiler chicks (Ross 308) were procured. All chickens were divided into 5 groups and raised under standard conditions. The groups were submitted to vaccinations against IBV and NDV, except for the non-vaccinated control group. Sera were collected at 18 and 45 days of age and submitted to serologic tests to assess antibody levels.

Vaccinia

Newcastle-Bronchitis Vaccine Cevac® , ND LaSota vaccine Cevac® , Hitchner B1 vaccine Cevac®, IB 4/91 Cevac®

Experimental design

The chickens divided into five equal groups (1 to 5) .The chickens in the groups 1 were kept as a control group and did not receive vaccine. The chickens in the groups 2 were vaccinated with commercial mixed vaccines (Newcastle-Bronchitis Vaccine Cevac®) twice at 1 day of age by ocular route in each bird and at 18 days of age by oral drop route. The chickens in the groups 3 were vaccinated with two separate vaccines simultaneously (Hitchner B1 vaccine Cevac® in left eye and IB 4/91 Cevac® in right eye) at 1 day of age by ocular route and also were vaccinated with the same vaccins at 18 days of age by oral drop (Hitchner B1 vaccine Cevac® at first and IB 4/91 Cevac® at second). The chickens in the group 4 were vaccinated with single ND vaccine (Hitchner B1 Cevac®) at 1 day of age by ocular route and also were vaccinated with the same vaccine at 18 days of age by oral drop. The chickens in the groups 5 were vaccinated with

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single IB vaccine (H120 Cevac®) at 1 day of age by ocular route and also were vaccinated with the same vaccins at 18 days of age by oral drop. Sera were collected at 18 and 45 days of age and submitted to serologic tests to assess antibody levels.

Blood collection and serological tests

Blood collections in the control group were carried out at 1, 18, and 45 days of age in order to assess maternal antibody levels against IBV and NDV. The other groups were submitted to blood collections at 18 and 45 days of age. Blood samples were drained from the brachial vein and sera were separated, identified and frozen at -20˚ C until the serological tests were performed. Serum samples were analyzed by Hemagglutination inhibition test (HI) to detect antibodies against NDV according to Alexander et al. (1983) and by a commercial indirect ELISA (enzyme-linked immunosorbent assay) to detect anti-IBV antibodies (Alexander et al., 1983).

Commercial ELISA

commercial ELISA assay were performed in u-bottomed 96-well microtiter plates with commercial ELISA Kit (Bio Chek co.).

Microlplate heamagglutination inhinition (HI) assay

Beta procedure of micro-plate HI test was performed in U-bottomed 96-well microtiter plates with 5% chicken erythrocytes to determine the antibody level of the sera samples collected from the chicks of different groups. The test was conducted using constant 8HA unit ND virus and diluted.

Statistical analysis

The titers obtained by ELISA and HI were submitted to analysis of variance using the Statistical Package for social Sciences (SPSS) version 18.0 program .One Way ANOVA LSD Test were performed to determine the significant differences in HI and ELISA titres of chickens of each group after primary and secondary vaccination. Means were compared at a significance level of 5%.

Results

Antibody Responses to NDV

The highest Ab levels noted to NDV in the groups 3 were vaccinated with two separate vaccines simultaneously (Hitchner B1 vaccine Cevac® in left eye and H120 Cevac® in right eye) at 1 day of age by ocular route and also were vaccinated with the same vaccins at 18 days of age by oral drop (Hitchner B1 vaccine Cevac® at first and H120 Cevac® at second). Although difference between group 2 and 3, difference between group 2 and 4 and difference between 3 and 4 is not significant. Also in group 2, 3 and 4, difference between first and second response to vaccine against NDV is significant ($p<0.05$).

Antibody Responses to IBV

The highest Ab levels noted to IBV in the groups 2 were vaccinated with commercial mixed vaccines (Newcastle-Bronchitis Vaccine Cevac®) twice at 1 day of age by ocular route in each bird and at 18 days of age by oral drop route. Difference between group 1 and 2, 1 and 3 and between group 1 and 5 is significant at 45 days of age. Difference between groups 2 and 5, group 3 and 5, and difference between group 2 and 3 is not significant at both 18 and 45 days of age. In the group 2, 3 and 5, difference between first and second response to vaccine against IBV is significant ($p<0.05$).

In the present study, Comparison of The effect of commercial mixed live vaccines of Newcastle Disease and Infectious Bronchitis and the use of two separate vaccines given simultaneously on systemic antibody responses in chickens was performed using different vaccines of Newcastle Disease and Infectious Bronchitis. They include commercial mixed live vaccines and two separate vaccines given simultaneously and routes employed were ocular and oral drop. Overall consideration among the vaccinated groups it was observed that the HI titres of birds against NDV between group 2 that receives Newcastle-Bronchitis Vaccine Cevac® twice at 1 day of age by ocular route in each bird and at 18 days of age by oral drop route and group 3 that receives two separate vaccines simultaneously (Hitchner B1 vaccine Cevac® in left eye and

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Table I. Mean log2±SD of antibody titre in broiler against NDV detected by HI assay.

Group	**age of Blood collection**		
	0	18	45
1 (control)		1. 2±0.13[a]	Not detected [a]
2 (commercial mixed vaccines)	7.45±0.3	3.79±0.2[b]	5.86±0.19[b]
3 (two separate vaccines simultaneously)		4.25±0.16[b]	6.66±0.15[b]
4 (single ND vaccine)		3.9±0.09[b]	5.9±0.17[b]
5 (single IB vaccine)		-	-

SD=Standard deviation
Data points labeled with the same letter in each column (a–b) are not significantly different and data points labeled with the different letter in each column (a–b) are significantly different ($p<0.05$).

Table 2. Mean±SD of ELISA titer in broiler chickens which were vaccinated against IBV detected by ELISA.

Group	**age of Blood collection**		
	0	18	45
1(control)		86.95±17[a]	44.07 ±91[a]
2 (commercial mixed vaccines)		172.11±124[a]	1063.6±712[b]
3 (two separate vaccines simultaneously)	3477.37±1406	262±220[a]	1012.28±241[b]
4 (single ND vaccine)		-	-
5 (single IB vaccine)		153±124[a]	1035±200[b]

SD=Standard deviation
Data points labeled with the same letter in each column (a–b) are not significantly different and data points labeled with the different letter in each column (a–b) are significantly different.

IB 4/91 Cevac® in right eye) at 1 day of age by ocular route and also were vaccinated with the same vaccins at 18 days of age by oral drop (Hitchner B1 vaccine Cevac® at first and IB 4/91 Cevac® at second) did not have significant statistical difference although groups 3 produced higher immune response than group 2. The present study also revealed that difference between first and second response to vaccine against NDV is significant in all vaccinated group ($p<0.05$).

Discussion

It is concluded that since preparation and using of commercial mixeded vaccines (Newcastle-Bronchitis Vaccine Cevac®) is easier than preparation and using of two separate vaccines simultaneously (Hitchner B1 vaccine Cevac® and IB 4/91 Cevac®), thus administration of live commercial mixed vaccines is suitable and repeated vaccination can produce higher immune response against NDV. Considering

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among the vaccinated groups it was observed that the ELISA titres of birds against IBV between group 2 that receives Newcastle-Bronchitis Vaccine Cevac® twice at 1 day of age by ocular route in each bird and at 18 days of age by oral drop route and group 3 that receives two separate vaccines simultaneously (Hitchner B1 vaccine Cevac® IB 4/91 Cevac® in right eye) at 1 day of age were not statisticaly significant also those vaccinated with the same vaccins at 18 days of age by oral drop (Hitchner B1 vaccine Cevac® at first and IB 4/91 Cevac® at second) were not significant statisticaly although groups 2 produced higher immune response than group 3. The present study also revealed that difference between first and second response to vaccine against IBV is significant in all vaccinated groups ($p<0.05$). Also it concluded that administration of live commercial mixed vaccines is better and repeated vaccination can produce higher immune response against IBV. These results demonstrate that there is not any significant statistical difference between the vaccinated groups.

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