



Effect of Associated Vaccines on the Interference between Newcastle Disease Virus and Infectious Bronchitis Virus in Broilers

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ABSTRACT

The phenomenon of viral interference between live vaccines against Newcastle Disease and infectious bronchitis has been reported since the 50's and many researchers have reported its prejudicial effects on avian immunization. Therefore, this study evaluated the effect of associated vaccines on the interference between Newcastle disease virus (NDV) and infectious bronchitis virus (IBV) in broilers. There were 400 broiler chicks divided into five groups. The groups were submitted to mono or polyvalent vaccinations against IBV and NDV, except for the non-vaccinated control group (CG). Sera were collected at 35 and 45 days of age and submitted to serologic tests to assess antibody levels. It was observed the occurrence of interference in the immune response against NDV by the use of associated vaccines to NDV and IBV; however, the group that was immunized with commercial combined vaccines (IBV+NDV) presented antibody titers to NDV similar to the group that was given only vaccine against NDV. We concluded based on these preliminary studies that the interference of IBV on the immune response against NDV depends also whether the association between the two vaccines is done just before vaccination or in the manufacturing laboratory.

INTRODUCTION

Newcastle Disease (ND) and infectious bronchitis (IB) are important diseases in the poultry industry and cause great losses (Tu *et al.*, 1998; King & Cavanagh, 1991). 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. Earlier studies have reported that infectious bronchitis virus (IBV) interferes with the immune response against Newcastle disease virus (NDV) (Raggi & Lee, 1964; Bracewell *et al.*, 1972; Thornton & Muskett, 1975).

Nowadays, Gelb *et al.* (2004) restart discussions about viral interference by means of evaluating IBV and NDV replication using a new detection technique, the Reverse Transcription Polymerase Chain Reaction (RT-PCR). 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.

Material and Methods

Birds

The study was performed in experimental broiler houses at the



Veterinary College of Ceara State University. There were 400 one-day-old chicks from breeders aged 42 weeks. The birds were not vaccinated in the hatchery and were raised until 50 days of age at a density of 10birds/m². Standard management procedures were provided, including ad libitum water and feed.

Treatments and bird vaccination

The birds were divided into five experimental groups (n= 80 birds/group): a non-vaccinated control group (CG) and groups vaccinated at 8 days of age (G1, G2, G3-ABV and G3-CVLP). G1 and G2 were vaccinated against IB and ND, respectively. G3-ABV was given IB and ND vaccines combined just before vaccination. G3-CVLP was vaccinated using a commercial IB-ND vaccine that is combined during manufacturing.

Immunization was performed through instillation of 0.03 mL by ocular route in each bird. All vaccines were obtained from the same laboratory and vaccinations were performed by the same workers. ND-vaccines administered to G2, G3-ABV and G3-CVLP were prepared using the strain HB1 (titer 10^{6.5}) while G1 and G3-ABV-immunization against infectious bronchitis was performed with the strain H₁₂₀ (titer 10^{3.5}). G3-CVLP was given an IB vaccine prepared with the strain Massachusetts Ma5 (titer 10^{3.5}).

Table 1 - Experimental treatments.

Group	Vaccination (8 days of age)	Strain
GCNone	None	
G1IBV	H ₁₂₀	
G2NDV	HB1	
G3-ABV	IBV+NDV (associated just before vaccination)	H ₁₂₀ + HB1
G3-CVLP	IBV+NDV (combined at the manufacturing facility)	Ma5 + HB1

Blood collection and serological tests

Blood collections in the control group were carried out at 1, 25, 35 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 35 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.

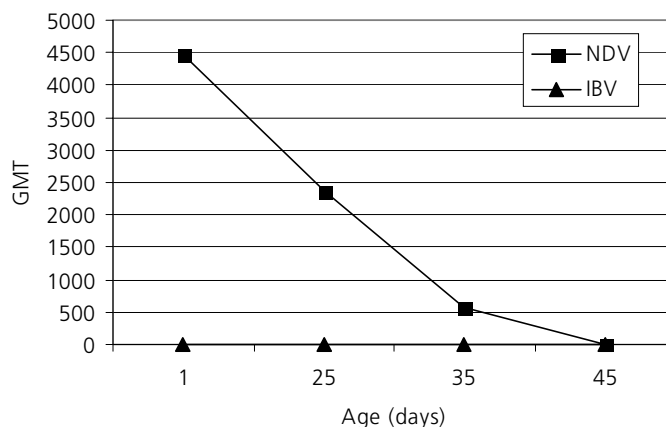
Serum samples were analyzed by Hemagglutination inhibition test (HI) to detect antibodies against NDV according to Alexander *et al.* (1983) and by a commercial indirect ELISA (enzyme-linked immunosorbent assay) to detect anti-IBV antibodies (Kirkegaard & Perry Laboratories – KPL).

Statistical Analyses

The titers obtained by ELISA and HI were submitted to analysis of variance using the statistical package SAS (SAS, 1999). Data on antibody titers were analyzed after logarithmic transformation (Log₁₀ x+1). Means were compared using the Student's t test at a significance level of 5%.

RESULTS AND DISCUSSIONS

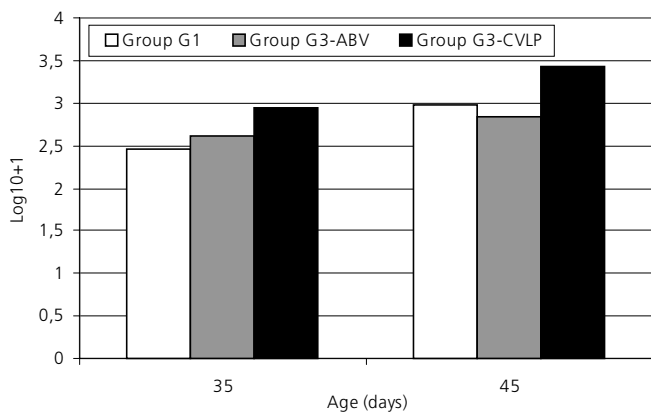
The control group presented antibody titers against NDV that decreased from GMT 4500 in the first day to basal levels at 45 days of age. Antibody titers against IBV were null throughout the experiment. Graph 1 shows NDV and IBV antibody curves of the control group.



Graph 1 - Titters of antibodies against Newcastle disease virus and Infectious bronchitis virus in the control group.

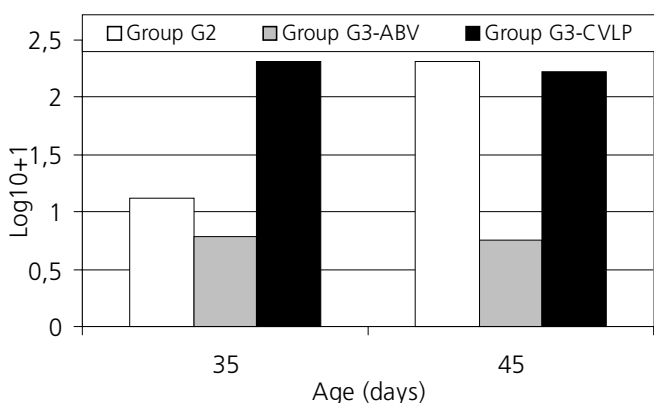
The progressive decrease in antibody titers against NDV until basal levels is in accordance to findings reported by Gelb *et al.* (1998) who showed that maternal antibodies decreased to approximately zero after few weeks. The reduction in NDV antibodies and the absence of IBV antibodies indicated that there was no IBV or NDV field challenge during the experiment. Graph 2 shows the antibody titers against IBV of groups G1, G3-ABV and G3-CVLP at 35 and 45 days of age.

Anti-IBV antibody titers were similar among the groups G1, G3-ABV and G3-CVLP; however, there was a small increase in titers in group G1 in relation to group G3-ABV and this difference was not statistically significant (p>0.05). Antibody titers against IBV in groups G1, G3-ABV and G3-CVLP were not different, showing that the immune response against IBV was not modified by NDV, results that are in accordance with Raggi & Lee (1964) and Zygraich *et al.* (1973).



Graph 2 - Antibody titers against Infectious bronchitis virus of groups G1, G3-ABV and G3-CVLP at 35 and 45 days of age.

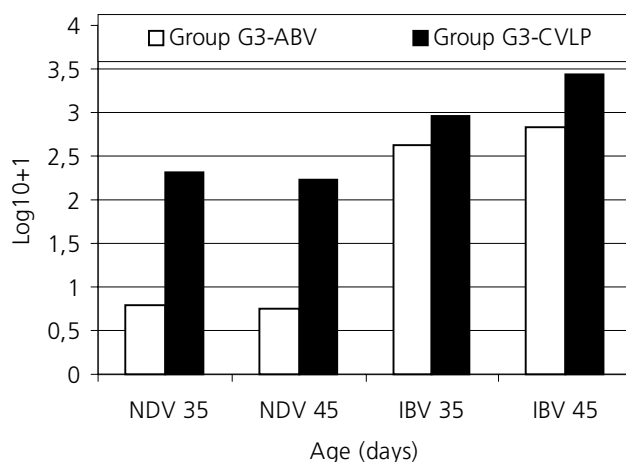
Graph 3 shows antibody levels against NDV of groups G2, G3-ABV and G3-CVLP at 35 and 45 days of age.



Graph 3 - Antibody titers against Newcastle disease virus of groups G2, G3-ABV and G3-CVLP at 35 and 45 days of age.

It is possible to observe an increase in the antibody titers of G2 at 35 to 45 days, although not statistically significant ($p > 0.05$). Group G2 was given only the vaccine against ND and presented higher antibody titers than G3-ABV, which was given a combined ND-IB vaccine. The low level of NBV antibodies in group G3-ABV might be explained by the interference between IBV and NDV. Many studies have reported this situation (Beard, 1967; Raggi & Lee, 1963; Raggi & Pignattelli, 1975; Yachida *et al.*, 1986). According to Gelb *et al.* (2004), the interference between IBV and NDV occurs because both of them infect initially the epithelial cells of respiratory tract and then replicate in the cell cytoplasm. Studies performed by Montgomery *et al.* (1997) using combined or not IBV and NDV vaccinations showed that IBV vaccination cause a decrease in the capacity of the Harder gland (HG) to

respond to antigenic stimulus. Therefore, the lower immune response by HG induced by IBV may then decrease anti-NDV antibody levels if vaccination has been performed with IB-ND combined vaccines. Cook *et al.* (2001) reported that IBV not only interferes with NDV, but also that there was interference with another paramyxovirus, the avian pneumovirus. Graph 4 shows antibody titers to IBV and NDV at 35 and 45 days of age in the groups that received associated vaccines (G3-ABV and G3-CVLP).



Graph 4 - Antibody titers against Newcastle disease virus and Infectious bronchitis virus in the groups submitted to associated vaccination.

Group G3-ABV presented lower IBV antibodies than the group G3-CVLP at 35 and 45 days of age; however, this difference was not statistically significant. This result shows that the fact of associating vaccines just before vaccination (group G3-ABV) or in the producing laboratory (group G3-CVLP) does not modify significantly the immune response against IBV. An opposite situation occurred regarding the immune response against NDV, because it was significantly modified by the use of the vaccines that were combined just before vaccination or those that had been combined in the producing laboratory. Group G3-ABV was immunized with the vaccines associated just before vaccination and showed NDV antibody titers that correspond to a situation of viral interference between IBV and NDV, because titers were lower than the titers shown by the group that was vaccinated only against ND (G2). Group G3-CVLP was given the commercial ND-IB-associated vaccine and presented NDV antibody titers similar to G2, demonstrating that there was no interference with the immune response. Some researchers have reported that there is no



interference between IBV and NDV (Zygraich *et al.*, 1973 and Winterfield, 1984) and in our experiment it was observed that interference did not occur in the group that was given the IBV+NDV vaccine combined in the laboratory. This finding is very relevant because many producers use vaccines associated just before vaccination, what might be promoting viral interference; on the other hand, interference does not seem to occur when vaccines combined in the producing laboratory are used, as observed in our experiment. Therefore, the use of vaccines associated in the laboratory is a viable option because viral interference does not occur uniformly in the vaccinated flocks (Smith, 2002). Consequently, part of the birds are infected with the vaccinal strain of NDV and the viruses are then transmitted to the birds in which IBV interfered with the immune response against NDV, promoting an increase of the strain virulence by the reverse transmission of the vaccine. As a consequence, interference can result in high levels of stress, low production and an insufficient immunity against field strains of NDV. More studies should be performed to better understand the factors that inhibited the interference of IBV on the immune response against ND vaccine.

CONCLUSION

This study shows, as preliminary results, that polyvalent vaccination with vaccines associated before vaccination or in the laboratory during manufacturing is a factor that interferes on the development of the immune response against NDV or on the virus itself. The results indicate that the use of polyvalent vaccines (IBV+NDV) combined in the manufacturing laboratory can attenuate the interference between these viruses when compared to vaccines associated just before vaccination.

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