Area Newcastle

COMPARATIVE EFFICACY OF TWO COMBINED CLONED VACCINES AGAINST NEWCASTLE DISEASE VIRUS (NDV) AND INFECTIOUS BRONCHITIS IN A CONTROLLED NDV CHALLENGE IN LAYING HENS.

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1 INTRODUCTION

Newcastle disease virus (NDV) and infectious bronchitis (IB) are important factors causing direct and indirect financial losses in batches of laying hens. The control of both diseases is achieved mainly through vaccination, which is practiced by egg-producing companies in many countries. In order to reduce costs, the combined vaccination against NDV and IB became common practice in the poultry industry. Within the portfolio of available vaccines for NDV and IB control, those produced from LaSota strains with low pathogenicity and high immunogenicity are the most indicated for combined vaccination, in order to avoid severe vaccine reactions. For the prevention of IB, combined vaccines are made from live attenuated strains, such as H120 and Ma5, due to their ability to promote cross-protection for different IB serotypes (BIJLENGHA et al., 2004; MENDONÇA et al., 2009).

2 OBJECTIVES

This study comparatively assessed the safety and efficacy of two combined NDV/IB vaccines (**HIPRAVIAR® CLON/H120** and **a vaccine containing La Sota cloned/IB Ma5**) in commercial layers. Safety was assessed by determining post-vaccine reactions, and efficacy by evaluating the serological response and protection against the controlled challenge two weeks post-vaccination.

3 MATERIALS AND METHODS

The study was conducted at Chulalongkorn University in Bangkok, Thailand. For this experiment, 100 commercial chicks were housed from 1 day of age, with low levels of maternal antibodies against NDV. At 4 weeks of age, these birds were separated into 3 experimental groups: **Group 1 (G1)** with 39 birds, which were vaccinated by eye drop at 4 weeks old with a combined freeze-dried vaccine containing a strain cloned from La Sota strain of NDV (HIPRAVIAR® CLON/H120, dose ≥ $10^{6.5}$ EID_{50}) and the H120 strain of IB; **Group 2 (G2)** with 39 birds, which were vaccinated by eye drop at 4 weeks old with a combined freeze-dried vaccine containing ND (La Sota cloned ≥ $10_{6,0}$ EID_{50}) and IB (Ma5 strain); **Group 3 (G3)** with 22 birds, which were not vaccinated (negative control). At 6 weeks of age (2 weeks post-vaccination), 30 birds from **G1** and **G2** and 15 birds from **G3** were challenged via oral drop with NDV (genotype VII, dose 10^5 EID_{50}) (**Table 1**).

GROUP	NUMBER OF BIRDS	VACCINE AT 4 WEEKS	NUMBER OF BIRDS CHALLENGED (NDV/VII) AT 6 WEEKS
GROUP 1	39	(HIPRAVIAR® CLON/H120) Via eye drop	30
GROUP 2	39	(La Sota cloned/IB Ma5) Via eye drop	30
GROUP 3	22	Not vaccinated	15

Table 1. Experimental Design

The birds were monitored for clinical signs and mortality for three weeks post-challenge. Post-mortem examinations were performed on the dead birds and macroscopic lesions were recorded.
All birds were weighed before vaccination, two weeks post-vaccination and three weeks post-inoculation (4, 6 and 9 weeks of age). Serology tests (haemagglutination inhibition [HI] for NDV and enzyme-linked immunosorbent assay [ELISA] for IB) were performed at 4, 6 and 9 weeks of age. Serology tests were also performed on the first day of the study to measure maternal antibodies.

The following parameters were measured:

- Post-vaccine reactions;
- Weight of the birds;
- Post-challenge mortality;
- Serology by HI for NDV and ELISA for IB (IDEXX IBV Ab Test, IDEXX Inc., The Netherlands).

The results were analysed by ANOVA and the Least Significant Difference (LSD) test. The percentage mortality rate was calculated using Chi-Square values.

4 RESULTS AND DISCUSSION

From 3 to 7 days post-vaccination, only mild respiratory signs (sneezing) were detected (**Table 2**). In the vaccinated groups (**G1** and **G2**), a non-significant difference ($p>0.05$) was observed in the overall number of post-vaccination respiratory responses, which was higher in **G2**, with 6 more signs/reactions than **G1** (41.03% and 25.64%, respectively).

The growth of the birds, measured by body weight at 6 and 9 weeks of age (2 and 5 weeks post-vaccination), was not affected by the two NDV vaccines used (**Figure 1**), even after the NDV challenge given at 6 weeks of age (**Figure 1**). In the two vaccinated and challenged groups (G1 and G2), protection after the challenge was similar ($p>0.05$) and high (96.7%) (**Table 3**). However, the non-vaccinated group (G3) did not survive the challenge, and no weights are therefore recorded for that group at 9 weeks of age.

Table 2. Number (N) and percentage (%) of layers with respiratory reactions (sneezing), whether vaccinated or not at four weeks old

GROUP[1]	DAYS POST-VACCINATION							TOTAL	
	2	3	4	5	6	7	8	N[2]	%
GROUP 1	0	0	1	4	3	2	0	10	25,64
GROUP 2	0	1	2	6	4	3	0	16	41,03
GROUP 3	0	0	0	0	0	0	0	0	0

[1] G1 = vaccinated (HIPRAVIAR® CLON/H120)
G2 = vaccinated (La Sota cloned/Ma5)
G3 = not vaccinated
[2] total number of birds per group = 39

Table 3. Percentage (%) mortality of laying birds vaccinated or not vaccinated and challenged with NDV strain VII

GROUP	VACCINE AT 4 WEEKS	NUMBER OF BIRDS CHALLENGED (NDV/VII)	% PROTECTION
GROUP 1	HIPRAVIAR® CLON/H120	30	96,67
GROUP 2	La Sota cloned/IB Ma5	30	96,67
GROUP 3	Not vaccinated	10	0

Figure 1. Body weight of the birds (g) at 4 weeks (before vaccination), 6 weeks (before the challenge) and 9 weeks (5 weeks post-vaccination and 3 weeks post-challenge)

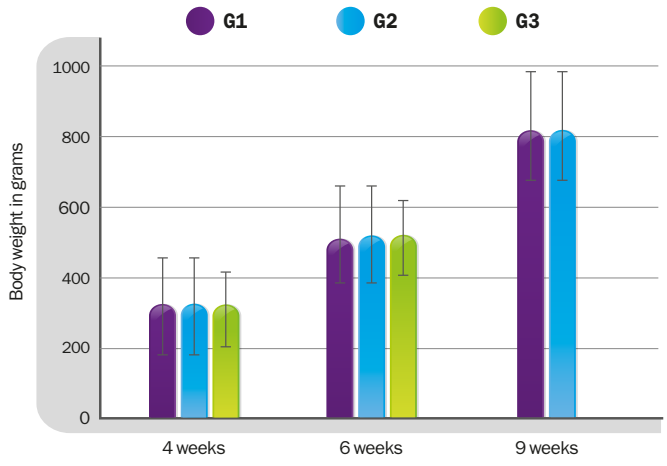
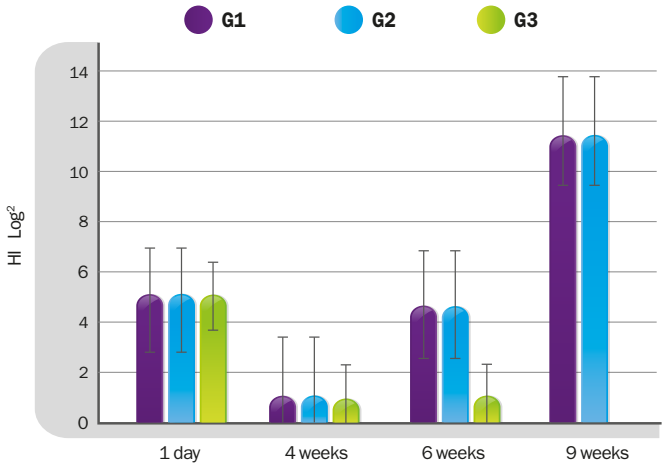
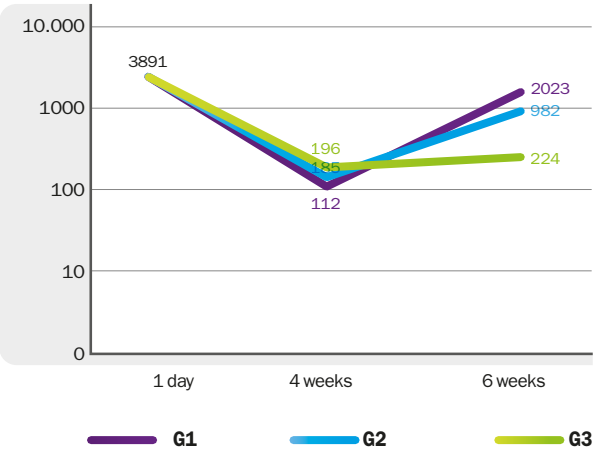


Figure 2. HI antibody titres (log^2) against NDV



Titres for maternal and post-vaccination NDV antibodies were established by HI (Figure 2), and the birds vaccinated with cloned NDV LaSota strains (**HIPRAVIAR® CLON/H120** or **La Sota cloned/Ma5**) were efficiently protected when they were challenged with NDV genotype VII (**Figure 2**). This was confirmed by the high antibody titres against NDV obtained after vaccination and the protection against the challenge in both groups G1 and G2, with no statistically significant difference between them ($p>0.05$). Significant differences ($p\leq0.05$) were observed in the ELISA serological monitoring of IB at 6 weeks of age (**Figure 3**). These differences can be explained by the type of IB vaccine used and the presence of maternal antibodies. Maternal antibodies can interfere with the immune response induced by the live vaccine, resulting in lower levels of, and more irregular, immunisation (JEON et al., 2008; GHARAIBEH & MAHMOUD, 2013), similar to the IB titre response obtained for the G2 group in this trial (**Figure 3**).

Figure 3. ELISA serology titres against IB



5 CONCLUSION

This experiment confirmed the efficacy and safety of the use of combined vaccines (**HIPRAVIAR® CLON/H120** or **La Sota cloned/Ma5**) for the control of Newcastle Disease in commercial layers against a controlled challenge with NDV virus genotype VII. Additionally, the results suggest milder post-vaccine reaction with the use of **HIPRAVIAR® CLON/H120**.

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