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## EFFICACY OF DIFFERENT INFECTIOUS BURSAL DISEASE VACCINES ADMINISTERED IN THE HATCHERY AGAINST A VERY VIRULENT IBDV CHALLENGE IN BROILER CHICKENS

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#### INTRODUCTION

An increasing trend towards vaccinating against IBDV in hatcheries using various technologies is evident, particularly with immune complex and recombinant vaccines. The aim of this study was to evaluate the protective efficacy of a combination of a next-generation immune complex vaccine (GUMBOHATCH<sup>®</sup>, HIPRA) with recombinant HVT-ND vaccines compared to double-insert recombinant HVT-IBD+ND vaccines against a challenge with a very virulent IBDV (vvIBDV). The study was also intended to assess whether the administration of GUMBOHATCH<sup>®</sup> interferes with the immune response produced by recombinant HVT-ND vaccines in broiler chickens.

		Early challenge		Late challenge	
		0 DPI	4 DPI	0 DPI	4 DPI
G1	BB	0.86±0.54 <sup>a</sup>	0.61±0.14 <sup>a</sup>	0.56±0.22 <sup>a</sup>	0.47±0.11 <sup>a</sup>
	HL	<b>3.20±0.84</b> <sup>a</sup>	2.07±1.16 <sup>a</sup>	1.40±1.14 <sup>a</sup>	1.27±1.16 <sup>a,b</sup>
	OB		<b>0</b> % <sup>a</sup>		0% <sup>a</sup>
G2	BB	0.67±0.05 <sup>a</sup>	0.64±0.30 <sup>a</sup>	0.78±0.58 <sup>a</sup>	0.50±0.16 <sup>a</sup>
	HL	2.40±0.55 <sup>b</sup>	2.00±1.36 <sup>a</sup>	2.60±0.89 <sup>b</sup>	1.47±1.25 <sup>a</sup>
	OB		<b>0</b> % <sup>a</sup>		0% <sup>a</sup>
G3	BB	2.52±0.51	1.95±0.58 <sup>b</sup>	1.76±0.28 <sup>b</sup>	1.74±0.37 <sup>c,d</sup>
	HL	0±0 <sup>c</sup>	$0.69 \pm 1.18^{b}$	0±0 <sup>c</sup>	0.60±1.30 <sup>b,c</sup>
	OB		33.33% <sup>b</sup>		6.67% <sup>a</sup>
	BB	2.49±0.64	1.83±0.54 <sup>b,c</sup>	1.99±0.59 <sup>b</sup>	1.54±0.54 <sup>b,d</sup>
G4	HL	0±0 <sup>c</sup>	2.67±1.63 <sup>a</sup>	0±0 <sup>c</sup>	2.73±1.67 <sup>e</sup>
	OB		73.33% <sup>c</sup>		33.33% <sup>b</sup>
G5	BB	2.55±0.45 <sup>b</sup>	1.63±0.40°	1.96±0.59 <sup>b</sup>	1.39±0.27 <sup>b</sup>
	HL	0±0 <sup>c</sup>	4.00±0 <sup>c</sup>	0±0 <sup>c</sup>	4.00±0 <sup>d</sup>
	OB		100% <sup>d</sup>		100% <sup>d</sup>
G6	BB	ND	2.00±0.45 <sup>b</sup>	ND	1.90±0.45 <sup>c</sup>
	HL	ND	0±0 <sup>b</sup>	0±0 <sup>c</sup>	0±0 <sup>c</sup>
	OB		0% <sup>a</sup>		0% <sup>a</sup>

## **MATERIALS & METHODS**

Day-old broiler chickens were obtained from a commercial source, distributed into groups (G) of 40 birds each and given different combinations of commercial vaccines subcutaneously, as indicated in Table 1. The chickens in G1 to G5 were subsequently challenged at either 28 or 35 days with 10<sup>5</sup> EID<sub>50</sub> vvIBDV virus by oral drop.

	Vaccination	vvIBDV challenge
G1	GUMBOHATCH® + HVT-ND recombinant vaccine A	28, 35 days
G2	GUMBOHATCH® + HVT-ND recombinant vaccine B	28, 35 days
G3	Recombinant HVT IBD + ND vaccine C	28, 35 days
G4	Recombinant HVT IBD + ND vaccine D	28, 35 days
G5	Non-vaccinated	28, 35 days
G6	Non-vaccinated	Non-challenged

Table 1. Experimental design

Clinical signs, body weight and mortality were monitored up to 4 days post infection (DPI). Necropsies were performed on the day of challenge and 4 days post-infection to evaluate macroscopic bursal lesions. A histopathological lesion score was given to the formalin-fixed bursal tissues on the basis of lymphoid necrosis and/or depletion according to Sharma, et. al. (1989). Bursal weights were also measured to obtain the bursal weight:body weight ratio (B/BW). Detection of the presence and identity of IBD in the bursa was performed by qPCR and partial VP2 gene sequencing. Spleen samples were collected at 28 and 35 days from 5 birds per group to detect the presence of HVT by means of qPCR. Blood samples were collected periodically to evaluate the immune response against IBDV and IBDV-VP2 by commercial ELISA test kits (Synbiotics-IBD Classic and Synbiotic-IBD Plus, respectively) and against the Newcastle Disease (ND) F protein of recombinant vaccines using a commercial ELISA test kit (BioChek). Body weight, B/BW ratios and antibody titres were analysed and compared between groups using ANOVA and least significant difference (LSD) test. Histopathological lesion scores (HLS) were compared between groups using the Kruskal-Wallis test. Significance was considered at p<0.05.

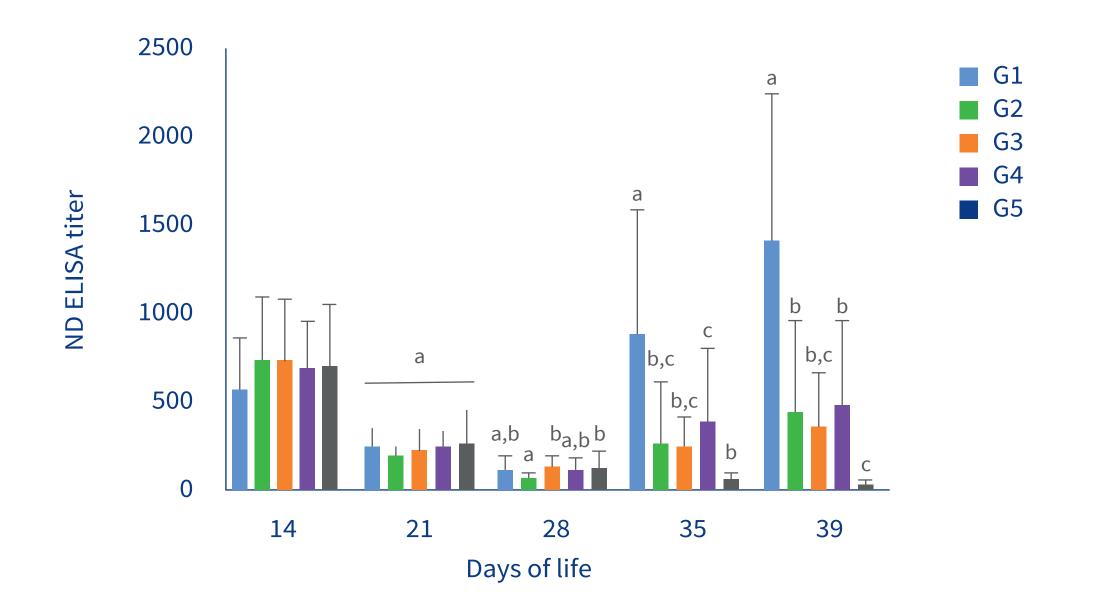
**Table 3.** Results of the bursa of Fabricius inspection after the two IBDV challenge experiments, at 28 and 35 days.

**BB:** mean ± standard deviation of the bursa to body weight ratio; **HL:** mean ± standard deviation of the histopathological lesion score; **OB:** percentage of oedematous bursae.

<sup>a,b,c,d</sup> The different superscript in each column indicate a statistically significant difference between groups (p<0.05).

Green and blue colours indicate that the GUMBOHATCH<sup>®</sup> strain (Hipra 1052) or the vvIBDV strain used for the challenge respectively were detected in the bursa of Fabricius. Orange colour indicates negative PCR results.

Each vaccine effectively stimulated an immune response against IBD prior to the challenge (data not shown); additionally, they also produced an immune response against ND (Fig. 1). PCR analysis revealed that despite the presence of antibodies, G3 and G4 failed to stop the replication of the challenge virus. In contrast, only the vaccine strain (Hipra 1052) was found in both G1 and G2 (Table 3). Finally, HVT was detected in spleens of all groups (100% of the birds at day 28 in G1 and G2, and at day 35 in G3 and G4), confirming the replication of the recombinant vaccines.



#### RESULTS

The results indicated that the challenge caused a growth delay in G4 and G5, especially with the early challenge (Table 2). G1 and G2 presented bursal atrophy before challenge, as expected with live IBDV vaccines. In addition, all the vaccines reduced the degree of bursal atrophy after the challenge compared to G5, but only G1 and G2 prevented macroscopic alterations, with a notable impact on oedematous lesions (Table 3). In contrast, there was a higher occurrence of these lesions with the G4 vaccine. Histopathological lesion scores were notably diminished across all vaccinated groups after receiving the challenge, with discernible variations, particularly evident in G4, where the reduction was comparatively less pronounced (Table 3).

Group	32 days	39 days	
1	409±45.73 <sup>a</sup>	388±32.88 <sup>a, b</sup>	
2	434±58.78 <sup>a</sup>	382±58.94 <sup>b</sup>	
3	408±29.68 <sup>a</sup>	366±51.24 <sup>b, c</sup>	
4	278±45.51 <sup>b</sup>	331±67.85 <sup>c</sup>	
5	282±54.54 <sup>b</sup>	239±74.14 <sup>d</sup>	
6	417±63.49 <sup>a</sup>	433±83.13 <sup>a</sup>	

**Figure 1.** Antibody response against ND F protein. Results are represented as mean and standard deviation of the ELISA titres. Different superscripts indicate a statistically significant difference between groups (p<0.05)

## **DISCUSSION AND CONCLUSIONS**

The results suggest that GUMBOHATCH<sup>®</sup> and the recombinant HVT ND vaccines can be combined without compromising the efficacy against IBD nor the immunization or the replication of the recombinant HVT ND vaccines. Additionally, GUMBOHATCH<sup>®</sup> showed better performances, when the efficacy was compared with recombinant HVT+IBD-ND vaccines. Notably, GUMBOHATCH<sup>®</sup> stopped the replication of the challenge virus in the bursa, whereas the recombinant vaccines did not, which emphasizes the importance of a live virus for competitive exclusion protection. It is important to highlight the fact that HVT-IBD-ND vaccines have a delayed onset of immunity, which could affect protection against early infections. In conclusion, the study suggests that combining GUMBOHATCH<sup>®</sup> with a recombinant HVT-ND vaccine is a safe and effective solution for IBDV control and bird immunization on high-risk farms.



#### deviation (SD) of the weight gain (g/bird).

#### <sup>a,b,c,d</sup> The different superscript in each column indicates a statistically significant difference (p<0.05).