EFFICACY EVALUATION OF A COMMERCIAL IMMUNE-COMPLEX VACCINE AGAINST THE EXPERIMENTAL CHALLENGE BY BRAZILIAN VERY VIRULENT G11 STRAIN OF INFECTIOUS BURSAL DISEASE VIRUS

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Introduction

Gumboro disease or Infectious Bursal Disease (IBD) is a viral disease that causes acute and highly contagious infection in young birds, affecting the lymphoid tissue of the bursa, which is responsible for their humoral immunity (ETERRADOSSI; SAIF, 2020). The infectious bursal disease virus (IBDV) belongs to the Birnaviridae family whose transmission is mainly by the oral route (FERREIRA, 2012). There are two serotypes of IBDV (serotypes 1 and 2), strains of serotype 1 are classified as classical virulent, very virulent, and antigenic variant, and these strains only affect chickens (ETERRADOSSI; SAIF, 2020; FRAGA et al., 2019). The acute form of the disease generally develops in animals up to 6 weeks of age, which may exhibit symptoms such as anorexia, prostration, depression, white diarrhea, ruffled feathers, and even death. In the case of the moderate form, loss of productivity, slow growth and secondary diseases may occur (KNEIPP, 2000; FERREIRA, 2012). In the world, the poultry industry has encountered heavy economic losses associated with very virulent (vv) IBDV strains in the last several years. In Brazil, episodes of high mortality were observed in mid-1997 caused by a IBDV from group 11 (G11), similar to the very virulent (vv) virus found in Europe (IKUTA et al., 2001; BOLIS et al., 2003). IBDV is very stable and resistant to many disinfectants, therefore vaccination is considered as the best way to control the disease (WAGARI, 2021).
The objective of this study was to evaluate the efficacy of a commercial immune-complex vaccine against the challenge by the Brazilian G11 strain of Infectious bursal disease virus (IBDV), and by the serological response.

Materials and Methods

The execution of the experiment was previously approved by the Ethics Committee on the Use of Animals (protocol 018/2022), and all the procedures and animal care were followed. 80-day-old Specific Pathogen Free (SPF) Leghorn chicks were housed in isolators (biological safety cabins), which have controlled air exhaustion and pressure. The birds were provided with age-appropriate feed and had *ad libitum* access to water during the experimental period. The birds were divided into 4 experimental groups: 1 vaccinated and challenged (GT1; n=20), 1 vaccinated and non-challenged (GT2; n=20), 1 non-vaccinated and challenged (GCP; n=10), and 1 non-vaccinated and non-challenged (GCN; n=20). 1-day-old chicks of GT1 and GT2 were vaccinated with Vaxxon IBD Imc (immunocomplex vaccine against IBD, Vaxxinova Brazil), subcutaneously, with 0.2 mL. Animals from GT2 and GCN were used for blood collection and serological analysis by ELISA (Kit IBD BioChek). Blood was collected on zero, 8, 14 and 21 after vaccination. On day 21 after vaccination, poultry of GT1 and GCP were challenged with a vvIBDV isolate, identified as a serotype 1 Gumboro disease virus of molecular group 11 (Simbios, Canoas, RS), by ocular route, with 10^3 $TCID^{50}$ /0.03 mL per bird. Mortality, body weight and bursa weight were registered until 5 days after challenge. The bursa of all chickens was collected for histological and molecular analysis. Histological analysis considered the lymphoid depletion lesions of bursa, and the score proposed by European Pharmacopoeia (v. 10.0) was used. Mortality and histologic bursa lesion score were analyzed by Chi-square test. The One-way ANOVA with Tukey's test was used to analyze body weight (BW), bursa weight (BuW) and the BuW/BW ratio. For serological analysis was used two-way ANOVA test with Sídák's posttest. A significance level of less than 5% ($P<0.05$) was adopted for all analyses.

Results and Discussion:

ELISA results demonstrated a high serological titer response in the vaccinated group (GT2), as shown in graph 1. After the challenge, poultry were evaluated for 5 days, but 100% of GCP chicks died before that, 3 days after the challenge, and all vaccinated and challenged poultry (GT1) stayed healthy and alive (Figure 01), as poultry of groups GT2 and GCN (non-challenged), significance difference was seen between groups ($p<0.0001$). These results corroborate with results reported by Bolis et al. (2003).
There was a high reduction in the bursa weight for the vaccinated groups (0.62 and 0.45 g) compared with the negative control (1.88 g) and positive control (1.12 g). The reduction in the bursa's size in the vaccinated groups occurs, because the vaccine is formulated with W2512 strain of IBDV, considered an intermediate plus strain, and its use is advantageous due to its ability to induce a response in the presence of higher maternal antibodies titers, compared to intermediate strains (SIMON; ISHIZUDA, 2000), however, this strain can cause greater damage and be more pathogenic to the bursa (CAMILOTTI et al., 2016). Body weight was significant lower for GCP poultry in comparation with GT1, GT2 and GCN. Graph 2 shows data from body weight, bursa weight and bursa weight-to-body weight ratio means, respectively. Statistical difference ($p<0.0001$) was seen in bursal histologic analysis, with lymphoid depletion score mean of 4.6 for GCP, 2.5 for GT1, 2.7 for GT2, and 1.0 for GCN. Was observed many bursae of GCP with hemorrhagic points (Figure 02), characteristic of challenge with G11 strain. Figure 03 shows histological cuts from bursa of GCN and GCP. Bursae were evaluated by PCR and results demonstrated 100% positivity for the G11 strain of GCP samples, with 1.42 x 10^7 DNA copies, and 80% positivity for genotype GM3-W2512 in GT1, with 3.93 x 10^3 DNA copies (Table 01). These results demonstrated the potency and efficacy of the immuno-complex vaccine tested.

Conclusion

The results demonstrated the high effectiveness of the Vaxxon IBD Imc vaccine, with a 100% survival rate among vaccinated and challenged poultry compared to 0% among non-vaccinated poultry. Additionally, there was a reduction in PCR positivity, indicating the predominance of the vaccine strain.

Graph 01. GMT of antibodies of the vaccinated group (GT2) and negative control group (GCN) at different evaluation time points prior to challenge. The dashed line (--) represents the cut-off of the ELISA kit. ns- no significant difference between groups ; ****significant statistical difference (p<0.0001).

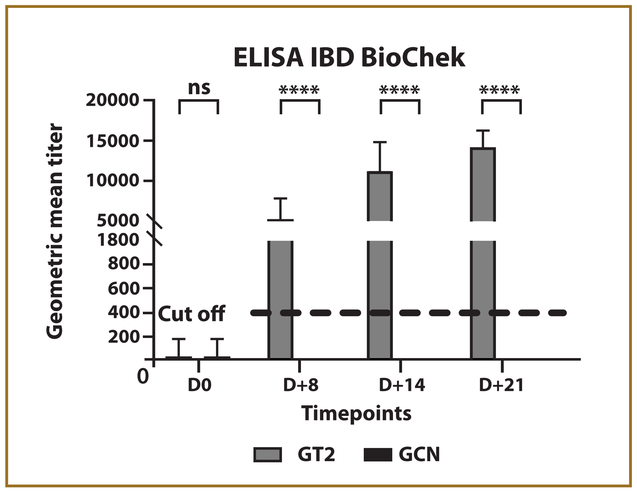
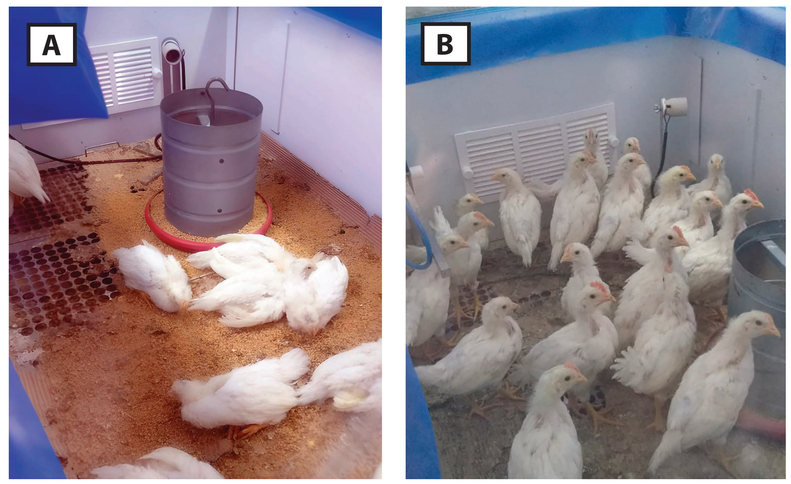


Figure 01. Dead poultry of the GCP group 3 days after the challenge (A), live poultry of the GT1 group 3 days after the challenge (B).



Graph 02. Mean Body Weight (A), Bursa Weight (B), and Bursa Weight-to-Body Weight Ratio (C) in Groups GCP, GT1, GT2, and GCN.

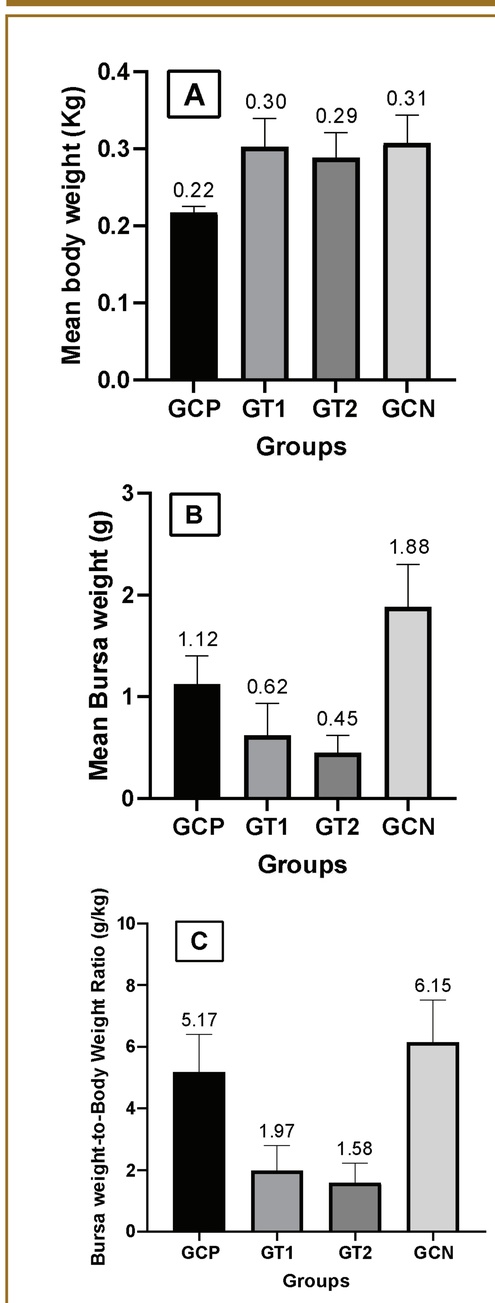


Figure 02. Hemorrhagic bursa from a bird of GCP group.

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Figure 03. Histological cuts from bursa. (A) Bursa from bird of GCN, lymphoid depletion score 1. (B) Bursa from bird of GCP, lymphoid depletion score 5.

Table 01. Result of detection, quantification, and genotyping of IBDV by PCR in the Bursae of birds of groups GCN, GCP, GT1 and GT2.

Group	Number of samples	Positive (%)	Viral quantification mean (DNA copies)	Genotyping
GCN	10	0	0 C*	-
GCP	10	100	1,42 x10^7 A	11
GT1	20	80	3,95 x10^3 B	GM3-W2512
GT2	10	60	3,12 x10^3 BC	-

*Different letters in the same column indicate significant difference between the groups ($p<0.05$), by Kruskal-Walli's test and Dunn's posttest.