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Journal Article

IMMUNOGENICITY STUDY OF INFECTIOUS BURSAL DISEASE VIRUS VACCINES IN COMMERCIAL BROILER CHICKENS

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SUMMARY

The infectious bursal disease (IBD) is a highly contagious viral disease affecting young chickens, causing bursal depletion and leading to economic losses where biosecurity and vaccination are considered the most effective control measures. This study compared the immunogenicity of the commercially available herpesvirus of turkey (HVT) vector vaccine (HVT+IBD) and a live attenuated plus IBD vaccine (IBD+) in commercial broiler chickens. Vaccines were administered to broilers per manufacturer's guidelines, where the HVT+IBD vaccine for day-old chicks subcutaneously and the IBD+ vaccine for day 12 via eye/oral drop. Serological responses were evaluated using IDEXX and IDvet ELISA kits. The broiler chickens had high maternally derived antibody (MDA) titers detected by both ELISA kits prior to vaccination. At day 28, both HVT+IBD and IBD+ vaccines elicited strong antibody responses, 5992.33 ± 3817.29 and 4775.00 ± 3418.77, respectively. High seroconversion antibody was detected from the IBD+ but not from the HVT+IBD vaccinated birds. Bursal histopathological analysis showed mild bursal lesion in the HVT+IBD vaccinated group 0.54 ± 0.44, whereas the IBD+ vaccinated group exhibited moderate bursal lesions (3.00 ± 0.22). Additionally, seroconversion of the HVT+IBD vaccinated chickens is associated with replication of the HVT vaccine in the bursa and spleen as detected by qPCR. This study showed while both vaccines can elicit high antibody titers, a successful vaccination of the HVT+IBD vaccine associated with high seroconversion and minimal bursal lesions, highlighting the importance of vaccine choice against IBD.

Keywords: ELISA, HVT+IBD, Infectious Bursal Disease Virus, Live Attenuated Vaccine, qPCR

INTRODUCTION

Infectious bursal disease (IBD) is an acute, contagious viral disease in young chickens (Eterradossi & Saif, 2020). The causative agent of this disease is the infectious bursal disease virus (IBDV). The virus was first detected in the USA, in 1962 (Cosgrove, 1962) , and in 1991 in Malaysia (Hair-Bejo, 1992). IBDV targets the bursa of Fabricius, destroying the developing B cells (Dey et al., 2019). IBDV has various strains that can cause dramatic economic losses by direct effects like high mortality or indirectly by immunosuppression which leads to secondary infections in the farms, depending on the virulence of the virus, the age, and the breed of the chicken (Dey et al., 2019).

IBDV is a single-shelled, double-stranded RNA virus from the Birnaviridae family. This virus is a non-enveloped virus, which is why it is extremely stable and can persist for 122 days in the farm environment and for more than 50 days in feed, feces, and water (MacLachlan & Dubovi, 2017). Farm husbandry and sanitary measures commonly used to poultry farms may not be sufficient to remove the virus. Hence vaccination is the most effective approach in controlling IBD (Ray et al., 2021). Presently, several different types of vaccines have been developed and used to control IBD in commercial chickens (Müller et al., 2012). However, due to the complexity of poultry farming and the diversity in the virulence and antigenic variations, there is no one standardized vaccination program to combat IBD in chickens.

Live attenuated vaccines are one the common group of vaccines that mimic infection in the target host (Müller et al., 2012). They are the most prevalently used and are classified as mild, intermediate and intermediate plus based on the virulence of the vaccine virus (Eterradossi & Saif, 2020). The difference between these vaccines is their ability to break through the maternally derived antibodies (MDAs) that can interfere with the vaccine virus (Eterradossi & Saif, 2020). In addition, a virulence vaccine virus is often used to control very virulent (vv) IBDV outbreaks in commercial farms (Rautenschlein et al., 2005; Thomrongsuwannakij et al., 2021).

The idea for vaccination is to boost the waning maternal antibody level and extend the protection in the field (Baxendale et al., 1980). To this end vaccinating the birds at the optimal time vaccinated flocks can induce high antibody levels against the IBDV. However, the presence of MDAs interfering with the live attenuated vaccine antigen (De Wit, 1998). Hence, an optimal time for IBD vaccination has been determined as the MDA starts to wean (De Wit, 1998). In addition, live attenuated IBD vaccines could cause bursal lesions and induce immunosuppression, however, in a well-managed farm this can be managed properly to reduce its impact on the chickens (Ingrao et al., 2013; Mazariegos et al., 1990;

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Thomrongsuwannakij et al., 2021). To address some of the limitations of live attenuated vaccines IBD vaccine, the recombinant vaccines, namely the herpesvirus of turkey (HVT) as a vector to carry the IBDV viral protein 2 (VP2) gene, were designed (Prandini et al., 2016). These vaccines can be administered via *in-ovo* route or subcutaneously in one-day-old chicks (Prandini et al., 2016). The vaccinated chickens produce anti-VP2 antibodies that will protect the chicken against IBDV as VP2 is the main viral capsid protein that elicit vaccine-induced immunity (Franciosini & Davidson, 2022; Prandini et al., 2016). The objective of this study was to compare the immunogenicity of commercially available live attenuated IBDV plus (IBD+) vaccine with HVT expressing the VP2 of IBDV (HVT+IBD) in broiler chickens.

MATERIALS AND METHODS

Vaccination of broiler chickens with IBD vaccines

A total of 36, one-day-old commercial broiler chickens (Ross breed) grade A were purchased from reputable source. Upon arrival, the chicks were randomly divided into three groups (12 chickens per group), and they were kept in separate, ventilated rooms at the Animal Room Facility (ARF) of the Faculty of Veterinary Medicine, UPM. The birds were kept in stainless steel cages (200cm x 100cm x 75cm) and provided feed and water ad libitum. The study was conducted according to the guidelines approved by the Institutional Animal Care and Use Committee (IACUC) under AUP number: UPM/IACUC/AUP-R034/2021.

A commercially available HVT+IBD vaccine and live attenuated plus IBD (IBD+) vaccine were purchased from the vaccine manufacturer company. The HVT+IBD group was vaccinated subcutaneously with 0.2 mL per dose containing at least 3.6 to 4.4 log10 PFU at one day old while the IBD+ group was given 0.1ml per dose (> 10^2 EID50) via the oral route at 12 days old as recommended by the manufacturer. The negative control group received phosphate-buffered saline (PBS).

Determination of IBDV antibody response

Serum samples were collected at one day old and at 12 and 28 days old. Serum samples from negative control were also included for all time points to confirm the seroconversion of the vaccinated birds. The IBDV antibody titers induced by the vaccinated groups were measured using two commercially available ELISA kits based overall IBD virus (IDEXX, USA) and IBDV VP2 protein (IDvet, France) as antigens, following the manufacturer's protocols.

Determination of bursa and spleen ratios and bursa score.

After sacrificing the chickens on day 28, their body weight, as well as the weights of the bursa and the spleen, were measured using a digital scale (US Solid, USA). A post-mortem examination was conducted to determine any organ abnormalities. The bursal and splenic ratios were calculated using the formula previously described by Sharma et al., (1989). The bursa score was calculated using a bursa meter (Sellaoui et al., 2012).

Detection of HVT load by real-time quantitative PCR
DNA extraction

Extraction of DNA from the bursal and spleen samples was performed using a Kylt kit (SAN Group Biotech, Germany) according to the manufacturer's protocol. The concentration and purity of the DNA samples were measured by a nanodrop Bio-Spectrophotometer (Eppendorf, Germany).

Real-time PCR detection of HVT

HVT detection in the bursa and spleen was confirmed by the duplex qPCR method, using the primers and probes previously described (Islam et al., 2004). The assay was optimized using HVT-positive samples provided by Dr. Tan Sheau Wei (Abadiah Lab, UPM Serdang), and a standard curve was established using tenfold dilutions of extracted DNA from an HVT-positive spleen. Viral load was then calculated using the formula "y = mx + c," where x represents the mean Cq value, m is the slope, and c is the y-intercept.

After establishing the standard curves, the normalized HVT values were determined for the bursa and spleen samples. The HVT values for the unknown samples were calculated by comparing the amount of the target viral gene (SORF1 of HVT) to the reference gene, [α2 (VI) collagen], using the formula provided below. These normalized values represent the HVT gene content adjusted according to the reference gene.

Normalized sample value (Ns) = Target gene /Reference gene for each sample

Histopathology

The bursa and spleen tissue samples of day 28 were processed using the standard histological procedure and stained with hematoxylin and eosin for the histopathological examination (Alturkistani et al., 2016). The bursa lesions were scored based on their degree of severity as described previously by Hair Bejo (2004).

Statistical Analysis

The statistical analysis was carried out using SPSS version 23. The data on body weight, antibody titer, BBW, and SBW ratios were combined to mean and standard deviation. One-way and two-way analysis of variance (ANOVA) with Tukey's post hoc test were then used to assess for group differences. A t-test was used to analyze the samples in two groups.

RESULTS

Antibody response against IBDV

The maternally derived antibody (MDA) of day-old chicks was randomly measured (n=10), and the mean antibody titer was an average MDA of 2848.85 ± 1013.87, and 16329.21 ± 1751.80 when measured using IDEXX and IDvet ELISA kits, respectively. HVT+IBD vaccine was administered at one day old, as the MDA does not interfere with the HVT+IBD vaccine. However, vaccination with the IBD+ vaccine was carried out at day 12 as the MDA is declined, based on the Deventer formula calculation (data not shown), where the MDAs were 665.23 ± 228.42 and 3587.00 ± 963.34 as detected by IDEXX and IDvet ELISA kits, respectively.

The IDvet kit detects the VP2 gene of IBDV with a cut-off value of 1342 and the titers exceeding this value are considered positive. In two vaccinated groups, the HVT+IBD vaccinated group had a higher antibody titer of 5992.33 ± 3817.29 followed by the IBD+ vaccinated group (4775.00 ± 3418.77) which were both significantly different from the negative control group ($p < 0.05$).

The IDEXX kit detects the whole IBDV with a cut-off value of 396. The IBD+ vaccinated group has a significantly higher antibody titer against the IBDV in comparison with the HVT+IBDV vaccinated group and negative control group with a mean antibody titer of 1623.00 ± 2031.13 ($p < 0.05$). However, the IDEXX kit failed to detect the antibody titer of the HVT+IBD vaccinated group where the antibody titer was not significantly different from the negative control group (Table 1).

Bursal and spleen ratios on day 28

No clinical signs were recorded after the vaccination by either of the vaccines. In addition, the body weight of the vaccinated and the negative control groups were not significantly different ($p > 0.05$). The bursa score and ratio of the HVT+IBD vaccinated group were not significantly different from the control group ($p > 0.05$). Similar results were also detected for the spleen ratio. The spleen results were the same as well, they were not significantly different from the negative control group ($p > 0.05$), confirming that the HVT+IBD vaccinated group does not cause significant changes after vaccination. On the contrary, the live attenuated IBD+ vaccinated group's mean score of the bursa (4.70 ± 0.95) was significantly smaller than the other groups ($p < 0.05$). In addition, the mean bursal body weight ratio for the IBD+ vaccinated group, 0.97 ± 0.69 was significantly lower compared to other groups ($p < 0.05$). The group vaccinated with IBD+ vaccine also had an increase in spleen size in comparison with the HVT+IBD vaccinated and the negative control group ($p < 0.05$), but the HVT+IBD vaccinated group did not have significant differences with the control group ($p > 0.05$) (Table 2).

Table 1: Mean ELISA antibody titer against IBD at 28-day-old chickens following vaccination.

Groups	Mean antibody titer ± SD	
	IDEXX ELISA	IDvet ELISA
Negative Control	$26.70^{c} \pm 48.67$	$714.90^{bc} \pm 652.46$
HVT+IBD vaccine	$376.08^{c} \pm 444.16$	$5992.33^{a} \pm 3817.29$
IBD+ vaccine	$1623.00^{ab} \pm 2031.13$	$4775.00^{a} \pm 3418.77$

[a] Denote significant differences with the negative control group, [b] Denote significant differences with the HVT+IBD group, [c] Denote significant differences with the IBD+ group

Table 2: Body weight, bursa and spleen ratios and bursa score of broiler chickens following vaccination with IBDV vaccines

Groups	Body Weight (mean ± SD)	Bursa Score (mean ± SD)	Bursa Ratio (mean ± SD)	Spleen Ratio (mean ± SD)
Negative Control	1695.50 ± 181.73	$6.70^{c} \pm 0.82$	$2.02^{c} \pm 0.57$	$1.06^{c} \pm 0.44$
HVT+IBD vaccine	1693.40 ± 192.55	$6.32^{c} \pm 0.82$	$1.85^{c} \pm 0.51$	$0.93^{c} \pm 0.34$
IBD+ vaccine	1621.70 ± 263.36	$4.70^{ab} \pm 0.95$	$0.97^{ab} \pm 0.69$	$1.35^{ab} \pm 0.34$

[a] Denote significant differences with the negative control group, [b] Denote significant differences with the HVT+IBD vaccinated group, [c] Denote significant differences with the IBD+ vaccinated group.

Histopathology of bursa and spleen

The histopathology of the bursa and spleen of the commercial broiler chickens on day 28 is presented in Figure 1. The bursal follicles of the control group were intact and contained a healthy population of lymphoid cells in both the cortex and medulla regions (Figure 1a). The vaccinated groups with the HVT+IBD vaccine showed mean lesion scores of 0.54 ±0.44 (Figure 1c). In the group that received the live attenuated IBD + vaccine, atrophy and degeneration of lymphoid follicles, and lymphocyte depletion of bursa of Fabricius (BF) with a mean lesion score of 3.00 ± 0.22 were observed (Figure 1e).

The spleen tissues of the chickens in the control group showed no lesions (Figure 4b). Similarly, the spleen

of the HVT+IBD vaccinated group was normal and not affected by the vaccination (Figure 4d). However, the IBD+ vaccine caused an increase in the white pulp zone and necrosis with eosinophilic debris (Figure 4f).

Quantification of HVT in lymphoid organs

Detection of HVT in the lymphoid organs was based on a relative quantitation approach where two sets of primer and probes against the target gene (ORF1 of HVT) and housekeeping gene [α2 (VI) chicken collagen] in a duplex assay. The copy number was computed using the formula "y = mx + c" [where x = mean Cq value, y = viral load, m = slope, and y-int =intercept]. The PCR efficiency (E) for the HVT is 94.8 %, and the correlation coefficient (R2) is 0.990, while for the reference gene, the E and the R2 were 95.4 % and 0.998, respectively. The log-linear standard curve shows the high accuracy and sensitivity of the application of inputs of 1000 ng/µl to 0.01 ng/µl and the amount of HVT in the bursa and spleen was determined based on the normalized values. The mean normalized value of HVT quantity in the spleen and bursa were 0.89 ± 0.04 and 0.58 ± 0.08 respectively.

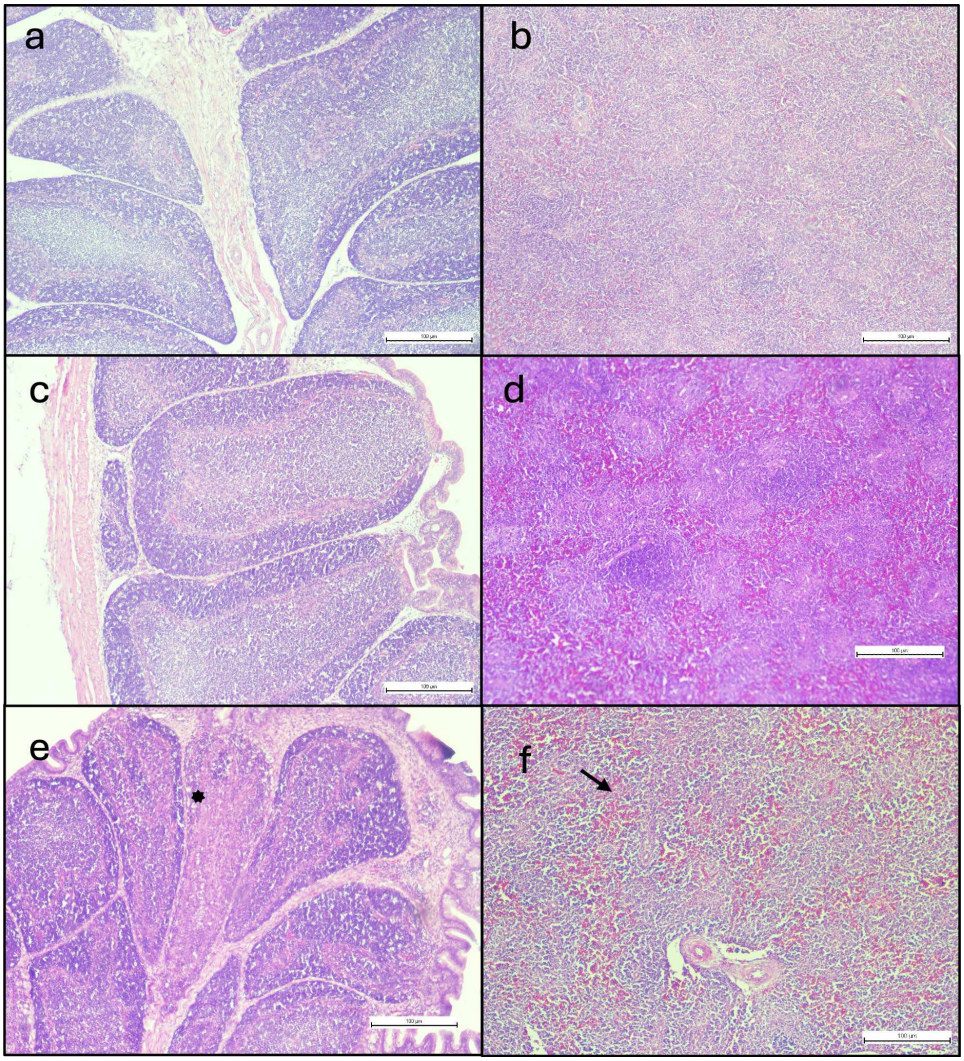

Figure 1. Histopathology of 28-day broiler chickens (H & E x 4). In the negative control group (a, bursa, and b, spleen), both organs were normal without lesions. In the HVT+IBD group (c, bursa, and d, spleen) the bursa and the spleen were also normal. The IBD+ vaccine (e, bursa and, f, spleen) degeneration of the bursa, hemorrhage (arrow) connective tissue thickening (asterisks), and spleen had increased zone of white pulps (arrow).

DISCUSSION

In this study, the immunogenicity of two commercially available vaccines was compared: A commercial HVT+IBD vaccine and a live attenuated IBD plus (IBD+) vaccine. Based on the best practices, the HVT+IBD vaccine was given at a day-old chick. The IBD+ vaccine was administered on day 12 according to the Deventer formula which is a vaccination system that determines the optimal vaccination time based on the MDA level (Block et al., 2007; De Wit, 1998). The final impact of both IBDV vaccines was evaluated on day 28. Serological measurements were obtained with two different ELISA kits: the IDEXX IBD Ab test kit, which used whole virions as antigens (Singh et al., 2010), and the IDvet ELISA kit which utilized VP2 protein of IBDV as antigens (Sedeik et al., 2019). As expected, high MDA against IBDV were detected in the commercial broiler chickens, which declined by day 28 in the non-vaccinated group. A previous study indicated that MDA level wanes

within 15-20 days post-hatching (Zahid et al., 2017). Both vaccinated groups showed significantly high antibody titer in comparison to the negative control group ($p < 0.05$). The IBD+ vaccinated group was seroconverted based on both ELISA kits. Since this is a live attenuated vaccine and MDA can neutralize it, the vaccination was performed at day 12 to induce optimum antibody titer at day 28, resulting in a robust immune response as reported in previous studies (Hamad et al., 2020; Sedeik et al., 2019; Thomrongsuwannakij et al., 2021).

In the HVT+IBD group, high antibody titers were detected using the IDvet ELISA, but not with the IDEXX ELISA. This outcome correlates with previous studies indicating that IDvet ELISA is a reliable serology assay to measure VP2-specific antibody levels following vaccination with HVT+IBD (Gewaily et al., 2023; Sedeik et al., 2019). ELISA is a reliable serology assay to detect antibodies against IBD and it correlates with protection (Marquardt et al., 1980); but differences in the antigens used to measure the antibody titers and the type of vaccine used may influence assay results and interpretation (Alhajj et al., 2023; Lemiere, 2012). Previously it was reported that the earliest antibodies to appear against the virus are against the VP3 protein (Fahey et al., 1989), which may partly explain why the IDEXX ELISA does not detect the seroconversion of the HVT vector vaccines containing the VP2 (Lemiere, 2012; Prandini et al., 2016). This highlights the importance of choosing a proper measurement kit to ensure reliable results and to set vaccination programs accordingly.

No clinical signs or mortalities were observed in the negative control or the vaccinated groups throughout the study, consistent with the previous research (Amer et al., 2008). Furthermore, histopathological examination showed no depletion in the lymphoid organs (bursa and spleen) of the HVT+IBD vaccinated group, confirming earlier studies (Kurukulasuriya et al., 2017; Sedeik et al., 2019). However, the live attenuated vaccinated group had both signs of bursal atrophy and microscopic bursal lesion compared to other groups, confirming similar findings by the previous studies (Amer et al., 2008; Rashid et al., 2013; Thomrongsuwannakij et al., 2021). Furthermore, bursal degeneration and an increase in the white pulp of the spleen in intermediate plus live attenuated IBD vaccine can cause immunosuppression that can negatively affect the overall vaccination program (Kumar et al., 2000; Roh et al., 2016). Regarding the spleen ratio, the study showed the HVT+IBD vaccine did not cause any significant changes in spleen size, whereas, the live attenuated vaccinated group had a significantly enlarged spleen (Kumar et al., 2000).

To further evaluate the immunogenicity of the recombinant IBDV vaccine, qPCR assay was used to assess the quality of HVT-based vaccine immunization. Detection of viral DNA of the vector vaccines is an important requirement to ensure the replication of the HVT+IBD vaccine (Lemiere, 2012). In this study, the HVT load in the spleen and the bursa was measured on day 28 to confirm successful vaccination. Higher protection induced by the HVT+IBD vaccine correlated well with higher vaccine virus genome load in lymphoid organs with stimulation of splenic T-cell responses (Baigent et al., 2005).

CONCLUSION

Both the vector, HVT+IBD and the live attenuated plus, IBD+ vaccines are able to elicit significant antibody responses. However, the choice of ELISA kits significantly influenced the detection of antibody titers, highlighting the importance of selecting appropriate diagnostic tools for accurate vaccine evaluation. The HVT+IBD vaccine showed lower lesion scores on the bursa, compared to the IBD+ vaccine, which was associated with bursal atrophy and potential immunosuppression. These findings underscore the critical need to carefully select vaccines and diagnostic methods to optimize vaccination programs, ensuring effective protection against IBDV while minimizing adverse impacts on flock health.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest

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