EVALUATION OF PROTECTION AGAINST T1-LIKE VARIANT INFECTIOUS BURSAL DISEASE VIRUSES USING DUAL RECOMBINANT HVT-ND-IBD VACCINE ALONE AND IN COMBINATION WITH LIVE INTERMEDIATE STRAIN AND 89/03 STRAIN OF IBDV VACCINE

Andres Montoya[A,C], Alexandra Reilley[A], Ivan Alvarado[A], Holly Sellers[B], Silvia Carnaccini[B]
[A] Merck Animal Health Rahway, NJ 07065 USA
[B] Poultry Diagnostic Research Center, University of Georgia, Athens, GA 30602 USA

[C]Corresponding author: E-mail: andres.montoya@merck.com

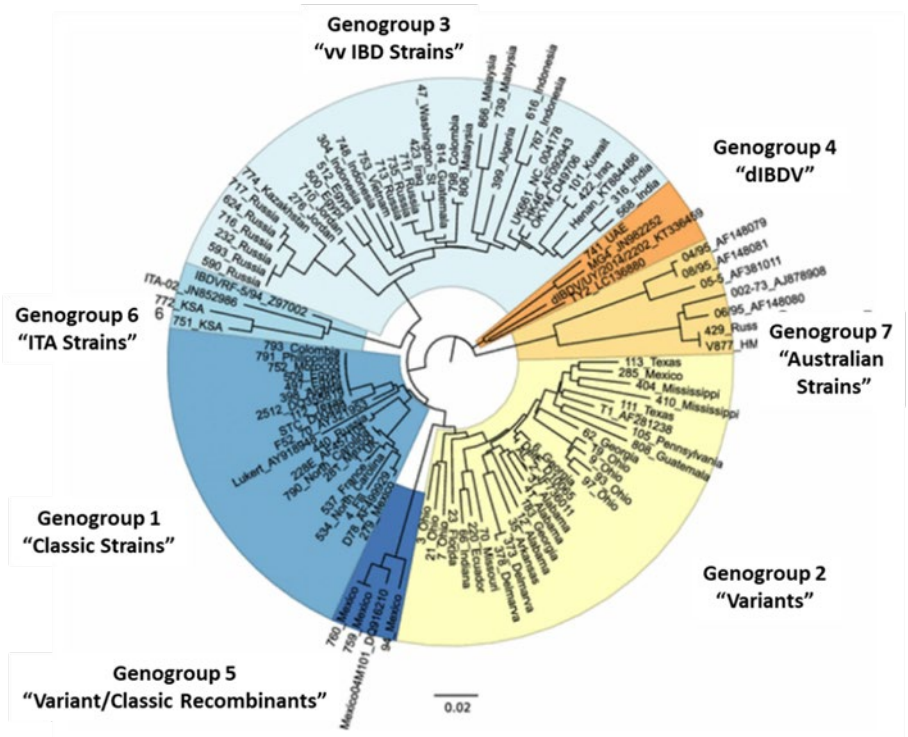
SUMMARY

Infectious bursal disease (IBD) is a highly contagious disease of young chickens caused by infectious bursal disease virus, characterized by immunosuppression and mortality generally at 3 to 6 weeks of age. IBDV is ubiquitous in commercial chicken operations. IBDV causes a prolonged B-lymphocyte immunodeficiency and increased susceptibility to various viruses and parasites. Both classic and variant strains of IBDV had been isolated in the southeastern United States. IBDV variant T1 belong to the sub-lineages G2B and like the AL-2 variant. Affect broilers between the ages of 20 to 30 days or sometimes older with moderate to severe bursal atrophy. IBDV Strain T1 variant had been reported to be the second most predominant IBDV variant in the USA present in broilers. An evaluation of the protection against IBDV challenge at 26 days of age with variant T1-IBD variant in SPF chickens vaccinated at day of age with HVT-ND-IBD with or without 89/03 and Live Intermediate Strain at 14 days of age showed, based on bursa/body weight ratio and histopathology, protection in all the vaccinated groups when it was compared with the non-vaccinated/Challenged group. Statistical difference was observed between the no vax/challenge group when compared to the vaccinated groups. The dual construct rHVT-ND-IBD vaccine alone or in combination with a live IBD vaccine protected against T1 like variant IBD virus when challenged at 26 days of age.

INTRODUCTION

Infectious bursal disease virus (IBDV) is present in poultry producing regions around the world and continues to be a major constraint for poultry producers. IBDV is a member of the genus Avibirnavirus in the family Birnaviridae, and its genome is composed of two segments of double-stranded RNA. The smaller segment B encodes VP1 and the larger segment A contains two partially overlapping open reading frames. The first, smaller open reading frame encodes a nonstructural protein VP5; whereas the second open reading frame encodes a precursor polyprotein, which is subsequently cleaved into VP2, VP4, and VP3. VP2 and VP3 are the major capsid proteins of IBDV. The VP2 protein has been identified as the major host-protective immunogen of IBDV and contains major epitopes responsible for eliciting neutralizing antibodies. One of the major consequences of IBDV is immunosuppression associated with vaccination failure and susceptibility of chickens to opportunistic pathogens. It was also shown that IBDV-infected birds may become a good propagator for other viral pathogens. Moreover, highly virulent IBDV can cause high mortality in unprotected flocks. IBDV replicates specifically in developing B-lymphoid cells, resulting in the destruction of the precursors of antibody-producing B cells in the bursa of Fabricius, and consequently, the immunosuppression. The first antigenic variant strain of IBDV was isolated from vaccinated flocks on the Delmarva Peninsula in 1985 and since then other variant strains were subsequently isolated in the United States and other countries. Before 1985 most typically isolated were the so-called classic isolates. Mainly in the southeastern of United States the most prevalent strains including classic and especially variants that are gaining grounds resulting in economic losses to poultry producers. IBDV variant T1 belong to the sub-lineages G2B and like the AL-2 variant. Affect broilers between the ages of 20 to 30 days or sometimes older with moderate to severe bursal atrophy. Sequence analysis performed by Dr. D. Jackwood in 2001, showed a close similarity of IBDV variant AL2 with an IBDV variant Tl strain (Graph 1). T1 IBDV variant had been isolated in broilers with recurrent respiratory problems and poor performance and also from broilers in several farms that present having poor performance during times of the year where downtime and broiler size will be critical for integrators. IBDV Strain T1 variant had been reported to be the second most predominant IBDV variant in the USA present in broilers chickens[1].

Besides biosecurity, vaccination is the most important measure to control IBDV in the field. The apparent inability to control IBDV infection through current vaccination warrants a necessity to develop alternate IBDV vaccine products and strategies that will improve the prophylactic measures vital to control IBDV. Molecular biology techniques have made it possible to use a recombinant vector vaccine to fight against two avian pathogens with a single vaccine. Turkey herpesvirus (HVT) is an excellent candidate for this objective. HVT is nonpathogenic in chicken, it has been widely used in chickens since the 1970s for inducing long-term cross-protection against Marek's disease, it is less sensitive to maternal-derived antibody than traditional attenuated IBDV vaccines and can be securely injected *in ovo* or subcutaneously in day-old chicks and presents no immunosuppressive effects. Bivalent recombinant HVT vaccines have been use commercially to protect against numerous diseases such as NDV, IBDV, ILT and AI. These recombinant HVT vaccines offer the advantage of inducing an immune response against Marek's disease as well as against a second disease by inserting a foreign gene in the vector that encodes a specific protein to stimulate a protective immune response. Now the first dual construct rHVT vaccine (Innovax®-ND-IBD) which contains an insertion of the F gene of Newcastle disease virus and the VP2 gene of infectious bursal disease virus to provide protection against three diseases with early onset of immunity in one single shot is available for the poultry industry.



Graph 1. Linda O. Michel, Daral J. Jackwood. Classification of infectious bursal disease virus into genogroups.2017

OBJECTIVE

Evaluated the protection against IBDV challenge at 26 days of age with variant T1-IBD variant in SPF chickens vaccinated at day of age with HVT-ND-IBD with or without 89/03 and Live Intermediate Strain at 14 days of age.

MATERIALS AND METHODS

A *subcutaneous* injection was used for injection of the chicks for each treatment group at hatch. A minimum of 500 mls of each vaccine at 1x concentration was needed for vaccination. 120 SPF embryos were used in the study and divided in 6 treatment groups (Graph 2). The eggs were purchased from Charles River SPAFAS and set in PDRC hatchery. The challenge viruses used in this study were the T1 IBDV field isolate 144843 case submission to PDRC. The viruses were expanded in 3-week-old SPF chickens prior to the start of the study, then titrated in chicken embryos. The viruses were diluted to the challenge dose of $10^{3.5}$EID50/dose in tryptose phosphate broth (TPB. Each bird in challenged treatment groups received 0.03 mls by the intraocular route of inoculation at 26 days of age. Birds were housed in negative pressure isolation units and have unrestricted access to feed and water. Birds were fed unmedicated Southern States All Grain Start-N-Grow diet.

The rHVT-ND-IBD and live IBDV vaccines were mixed in the same diluent when both were given at day of age. At 14 days of age two treatment groups received by intraoral route a live intermediate strain IBDV vaccine. Birds were challenged at day 26 of age with T1 IBDV field isolate via intra ocular route. The study was terminated on day 33 and birds and bursae weighed data for Bursa /Body Weight (Bu/BW) ratio were collected and bursas also were collected for histopathology to determine mean bursal lesion scores to determine atrophy (Graph 3 and Graph 4). Protection against challenge was determined by Bu/BW ratios greater than or equal to the Bu/BW ratio of the Unvaccinated Challenge Group + 2 standard deviations and represented by the dotted line in graph 2 and graph 4. The histopathology bursal lesion scoring system is based on scores of 0-4 where 0 is an absence of lesions and 4 is marked lymphoid atrophy (Pictures 1,2,3, and 4). Statistics were performed on the Bu/BW ratios using the unpaired t-test (GraphPad Prism, v. 6). Statistics were performed on the bursal lesion scores using the unpaired t-test (Graph Pad Prism). Different letters above the bars in the graphs denote groups with significant differences in bursal lesion scores.

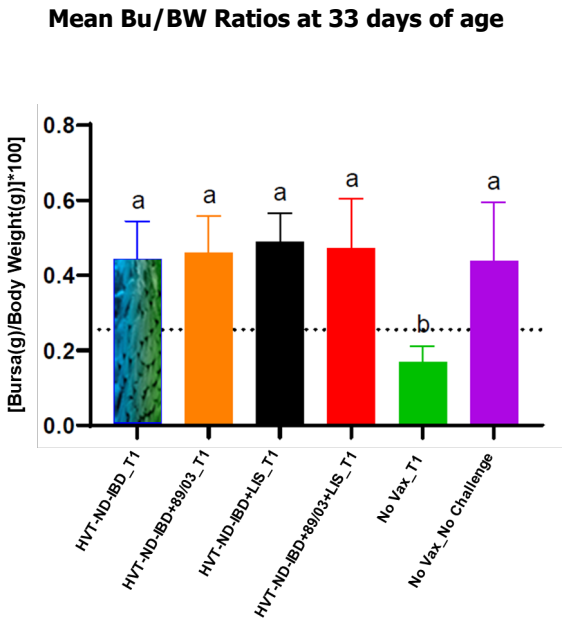
Group	No. of Chicks	SQ Day 1	14 day of Age IO[A]	Challenge IO[B] 26 doa[C]
1	20	HVT-ND-IBD		T1
2	20	HVT-ND-IBD + 89/03		T1
3	20	HVT-ND-IBD	Live Intermediate Strain IBD Vaccine	T1
4	20	HVT-ND-IBD + 89/03	Live Intermediate Strain IBD Vaccine	T1
5	20	No Vax		T1
6	20	No Vax		None

Graph 1. Different treatment groups. ([A]intraoral [B]intraocular, [C]day of age).

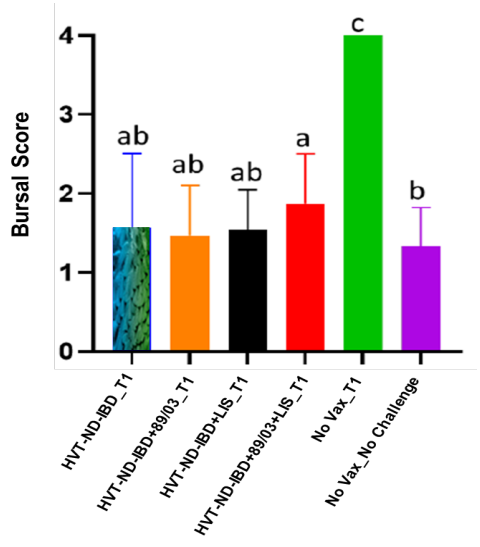
RESULTS AND CONCLUSIONS

Criteria for protection was based by bursa/body weight ration and histopathology. Results showed protection in all the vaccinated groups when they were compared with the Non-vaccinated/Challenged group. The Bu/BW ratios for all vaccinated/ T1 challenged groups were significantly higher at 33 days of age, compared to the NoVAXT1 group, providing evidence that all vaccine combinations and the Innovax®-ND-IBDV prevented significant bursal atrophy following T1 challenge. In addition, the Bu/BW ratios for all vaccinated/T1 challenged groups were greater than 2 standard deviations above the mean Bu/BW of the NoVAXT1 challenged group providing additional evidence for protection against challenge. The extent of lymphocytic depletion in the bursa was most significant in the NoVAXT1 group with a mean bursal lesion score of 4 compared to all other groups in this study. This confirms that the challenge virus and dose were valid. The bursal lesions scores for all vaccinated/T1-challenged groups were significantly lower compared to the NoVAXT1 group suggesting that all vaccine combinations provided protection against significant lymphocytic depletion in the bursa.

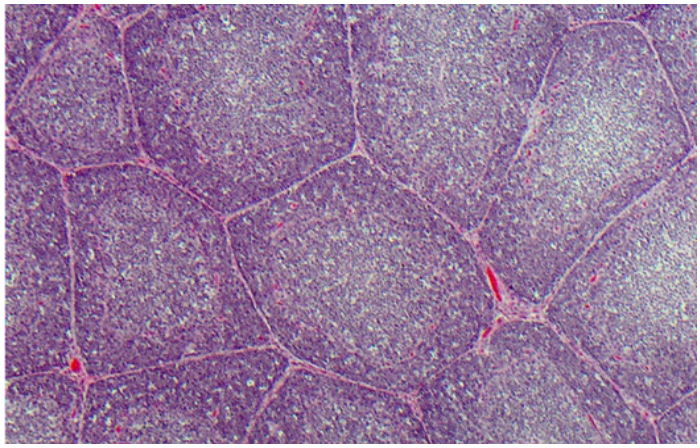
The Innovax®-ND-IBD alone or in combination with a live IBD vaccine protected against T1 like variant IBD virus when challenged at 26 days of age. These results add to previous work with Innovax®ND-IBD that showed protection in SPF birds that were vaccinated either by *in ovo* or subcutaneous route and challenged with IBDV STC APHIS (97% protection by the *in ovo* route and 100% protection by subcutaneous route) or with IBDV Variant E, AL-2, and 9109. In flocks with field challenges such as low maternal antibodies, uneven maternal antibodies or maternal antibodies that are not protective against field variant strain, or flocks that suffer immunosuppression and need to reduce shedding and field challenge, a combination of rHVT-ND-IBD with live IBDV vaccine at the hatchery or at 14 days of age may enhance protection against early challenge.



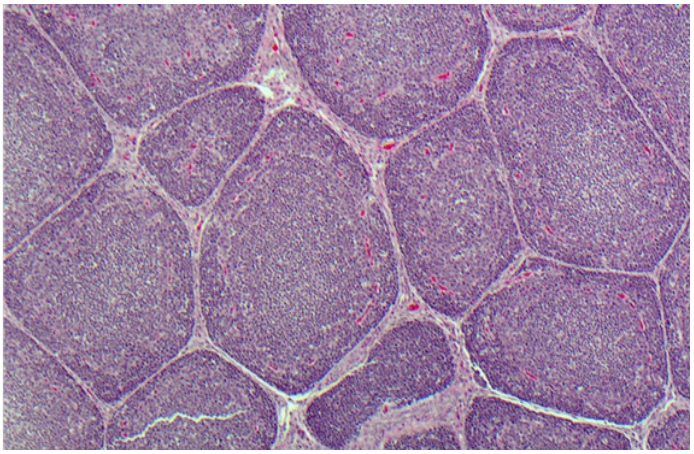
Graph 3. Mean bursal lesion score at 33 days of age post challenge with T1 IBDV field isolate.



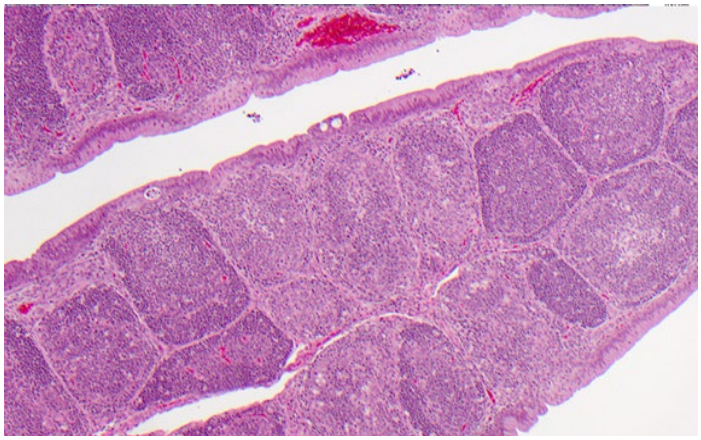
Graph 4. Mean bursal lesion scores at 33 days-of-age post challenge with T1 IBDV field isolate.



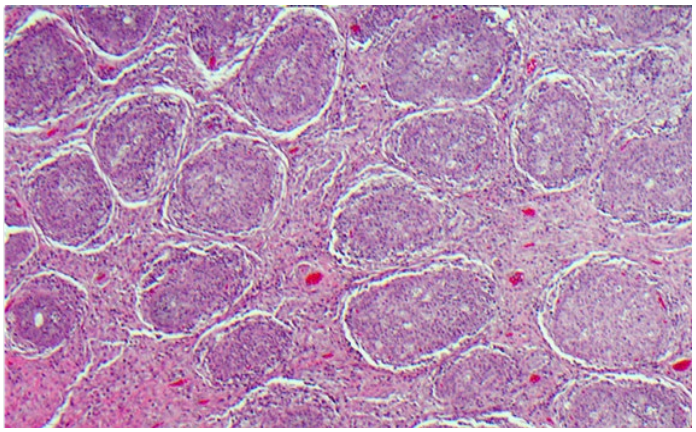
Picture 1. Score 1 <25% follicular atrophy (Mild lymphoid atrophy)



Picture 2. Score 2 25-50% follicular atrophy (Mild to moderate lymphoid atrophy)



Picture 3. Score 3 50-75% follicular atrophy (Moderate lymphoid atrophy)



Picture 4. Score 4 >75% atrophy (Marked lymphoid atrophy)

[1]Cookson K, et al. Update on IBD viruses infecting broilers today and how inactivated vaccines protect against different viruses in the AL2 family. 2020 Western Poultry Disease Conference.