

An update on Marek's Disease Vaccination, Diagnosis, and Immunosuppression

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#### THE AMERICAN ASSOCIATION OF AVIAN PATHOLOGISTS

promotes scientific knowledge to enhance the health, well-being, and productivity of poultry to provide safe and abundant food for the world.



## Boston, MA July 11-14, 2015

#### IBV 4/91 Infection in Ontario's Broiler Breeder Flocks: Clinical Signs, Pathologic lesions and test methods

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In early 2012 there was an increase in IBV cases in Ontario. Investigation involved genotyping of IBV samples submitted to the AHL between 2000 and 2013 and comparison of the nucleotide sequence of the spike (S) protein gene with sequences available in GenBank. Based on their S gene sequence similarities, Canadian IBVs could be divided into four groups: 1) classic, vaccine-like viruses, such as Connecticut and Massachusetts; 2) Canadian variant viruses, such as strain Qu mv; 3) US-like variant viruses, such as strains California 1734/04, California 99, CU 82792, Pennsylvania 1220/98, and Pennsylvania Wolg/98; and 4) non-Canadian, non-US viruses, such as strain 4/91. IBV strain 4/91had been previously identified in many European, African, and Asian countries as well as in Brazil and Mexico but had not been previously identified in Canada. The first IBV 4/91 positive sample in Canada occurred in 2011 and Ontario is currently the only province with confirmed cases of this IBV strain. The clinical signs, pathologic lesions and test methods for identification of IBV 4/91 positive cases in broiler breeders will be described.

#### Production of structural proteins of Avian Infectious Bronchitis virus by utilizing a novel yeast expression vector

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Infectious Bronchitis virus is one of the major diseases in the poultry industry, inflicting economic damage at a global scale. The viral genome of the virus is known to encode antigenic proteins S1 (Spike), S2, N (Nucleocapsid), E (Envelope), M (Membrane). Various efforts have been made to control and prevent outbreak of the disease but difficulties still continue up to the present day due to existence of various serotypes. Production of efficient vaccines was not very feasible as crossover protection between different serotypes of the virus was limited. Production of vaccine strains by attenuation is also not very efficient, due to the time consuming process. Nowadays, use of expression vectors has been introduced to produce vaccines efficiently, with expression systems utilizing various microbes such as bacteria, viruses, and yeast. Yeasts are known to be one of the most versatile expression systems, with various post-modulation capability and relatively easy to manipulate. In this study, we utilized a novel expression vector pYEGa-HIR25a and yeast strain Saccharomyces Cerevisiae Y2805 as our host expression system. The vector was processed to include the main antigenic proteins S1, S2, N, M, E of infectious bronchitis virus. Various vectors were constructed to express S1, S2 protein individually or produce Virus-like particles (VLP) by expressing N, M, E proteins along with S1 and S2. The vectors were inserted into competent E-Coli cells, and cultured 24 for hours before the vectors were extracted. At the present stage, the vectors including the S1 gene was confirmed by PCR analysis. While the exact level of antigenicity is yet to be confirmed, SDS-PAGE and western blotting showed possibility of successful expression of the viral protein. Further efforts are being conducted to analyze the level of antigenicity of constructed VLPs and S2 protein.

#### Avian Infectious Bronchitis Virus (IBV): Differential pathological and immunological profiles of two Brazilian variants.

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Two variant strains of IBV isolated in Brazil (3735 and 3736) have their pathological and immunological properties characterized in SPF chickens. Thus, H120 vaccinated or non-vaccinated groups were challenged with 10<sup>4</sup>EID<sub>50</sub>/bird of each IBV variant strain. At 1dpi, 5dpi and 8dpi, birds of each group were randomly necropsied. Tear and serum samples were quantified for IgG anti-IBV titers by ELISA, and tracheas were tested for microscopic lesions, viral load

and expression of innate (TLR3 and TLR7) and adaptive (CD8β, CD4) immune genes measured by RT-qPCR. TLR3 transcripts peaked at 1dpi, only in non-vaccinated groups and TLR7 transcripts increased significantly only in nonvaccinated birds challenged with 3735 strain. Memory humoral cross-immunity (serum and tear IgG anti-IBV) was elicited for both variant strains tested, while significant memory of cell-mediated (CD4 and CD8ß expression) crossimmune responses was induced by H120 vaccine strain only against 3736 variant strain. At 1dpi, no significant lesions were observed in the trachea, though the viral load was significantly increased in non-vaccinated and in the vaccinated group challenged with 3736 strain. At 5dpi, the lesions peaked only in non-vaccinated groups, and no differences were observed in the viral load for 3735-groups, but significantly reduced IBV copies were found in vaccinated group compared to non-vaccinated group when they were challenged with 3736 strain. Although the variants evaluated here caused similar tracheal lesions, they could stimulate different innate and adaptive immune mechanisms to control IBV infection, and 3736 strain, though from variant genotype, has a cross-immunity with H120 vaccine strain.

#### **Infectious Bursal Disease**

## Molecular Evidence of the Continued Evolution of Infectious Bursal Disease Viruses.

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Molecular analysis of infectious bursal disease virus (IBDV) strains from a number of countries worldwide has provided evidence for the continued evolution of this double-stranded RNA virus. Very virulent (vv)IBDV are most often identified in countries outside North America and are beginning to show some genetic heterogeneity within the hypervariable sequence region of VP2. Antigenic variant viruses are still predominately diagnosed in North America. A variety of amino acid mutations within the loop structures of VP2 were observed in these antigenic variants, suggesting that studies on the potential for antigenic drift are needed. Overall, the mutations observed

are often geographically restricted; indicating global dispersion of the viruses may be limited.

## Splenic gene expression profiling of chickens protected against infectious bursal disease by DNA vaccination

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Studies were conducted to compare splenic gene expressions of DNA-vaccinated chickens and unvaccinated chickens upon challenge by infectious bursal disease virus (IBDV) regarding innate immunity, immune cell regulation, and inflammation. One-day-old specific-pathogen-free (SPF) chickens were vaccinated intramuscularly three times at weekly intervals with IBDV large-segment protein (VP243)-expressing DNA. The chickens were challenged orally with IBDV strain variant E (VE) one week after the last vaccination. The spleens collected at various intervals after challenge were subjected to real-time **RT-PCR** quantification. The chickens challenged with IBDV only (group CC) had significantly higher (P<0.05) IBDV viral load in the spleens than those vaccinated with DNA vaccine and challenged with IBDV (group DC) at 3 and 5 days post challenge (dpc). The expression levels of innate immunityrelated Toll like receptor 3 (TLR-3) and melanoma differentiation-associated gene-5 (MDA5) in chicken spleens from group CC were significant higher than those in group DC at 3, 5, or 7 dpc. The expression levels of chicken IFN- $\gamma$  gene (Th1-related), IL-10 gene (T cell regulationrelated), and IL-1 $\beta$ , IL-6, IL-12, TNF- $\alpha$ , and IL-18 genes (proinflammatory and inflammatory cytokines) in chicken spleens from group CC were significantly higher (P<0.05) than those in group DC at 3, 5 or 7 dpc. In conclusion, the chickens protected from IBDV challenge by DNA vaccination do not have significantly enhanced and differential splenic gene expressions related to innate immunity, immune cell regulation, and inflammation.