

slot-blot test was performed in duplicate coating a nitrocellulose membrane with different dilutions of BoHV-1 (107 to 102 DICC/50ml). As negative control reaction the membrane was coated with BSA, while the positive control was purified immunoglobulin of serum of animals proved positive for BoHV-1. After coating, the membranes were inoculated with a solution containing the same immunoglobulins used as positive control (6.8mg/ml) overnight at 19° C and washes with PBST 0.05% were carried out shortly after inoculation of bovine IgG conjugated with colloidal gold. A strong mark was observed on the test with the lowest dilution (107) and in form of decreasing intensity, visualized to the dilution of 104. At the negative control lines no staining was observed, while in the positive control lines a strong coloring was visualized, thus validating the test performed. The results obtained demonstrate the feasibility of developing a quick test for lateral flow diagnosis of infected animals for the detection of antigens or antibodies, aiding in the diagnosis of infected or vaccinated animals. FINANCIAL SUPPORT: CNPQ EDITAL: MCT/CNPQ/FNDCT/FAPS/MEC/CAPES/PROCENTRO-OESTE Nº 031/2010.

VV194 - CELLULAR IMMUNE RESPONSE OF AVIAN INFECTIOUS BRONCHITIS VARIANT BY FLOW CYTOMETRY

Mattos, G.L.M.¹; Okino, C.H.²; Trevisol, I.M.²; Mores, M.A.Z.²; Brentano, L.²; Esteves, P.A.²; Lopes, L. dos S.²; Bastos, A.P. de A.²; Caron, L.F.³; Filho, T.F.³; Duarte, I.³; Montassier, H.J.

1. *Embrapa Suínos e Aves e Aluno de Doutorado no Programa de Pós Graduação em Medicina Veterinária. Área de Concentração Patologia Animal, da UNESP - Universidade Estadual Paulista, FCAV*

2. *Embrapa Suínos E Aves*

3. *Imunova Análises Biológicas e Universidade Federal do Paraná*

4. *UNESP - Universidade Estadual Paulista, FCAV, Departamento de Patologia Veterinária, Programa de Pós Graduação em Medicina Veterinária*

Avian infectious bronchitis virus (IBV) is an avian coronavirus that causes a highly infectious disease characterized by respiratory, reproductive and renal signs, depending on the viral tropism and results in a significant economic impact to the worldwide

poultry industry. Failures of vaccine protection on the field, may be related to incomplete cross-protection between the vaccine strain and the variant strains. The present work aimed to evaluate, immune-related markers (cluster of differentiation) in the peripheral blood of birds challenged with a Brazilian field isolate of IBV (F3735 strain), by flow cytometry. SPF birds divided into three groups of 9 animals were housed in positive pressure isolators. At one day old group A was vaccinated with attenuated vaccine strain (H120) of IBV. At 28 days of age, birds from both vaccinated group A and unvaccinated group B were challenged with 10^{4.0} EID₅₀/bird of the variant strain F3735. Birds belonging to group C were not vaccinated nor challenged (negative control group). Blood samples were collected from nine birds of each group on 5 days post-infection. Leukocyte surface antigens were assessed with specific monoclonal antibodies combined (CD4/TCR $\alpha\beta$ -V β 1/CD45; Kul-1/MHCII/CD45; CD8 α /CD28/CD45; and Bu-1a/CD45). Data were analyzed by analysis of variance using the MIXED procedure of SAS (2008) by testing the fixed effects of treatment. Comparison between means was made by Student's t test ($p \leq 0.05$). No significant differences were observed between groups A and C, and group B presented a significantly higher immune response of, CD4+TCR $\nu\beta$ 1+, CD4+TCR $\nu\beta$ 1- and CD8 α +CD28+ cells. The subpopulations CD4-TCR $\nu\beta$ 1+, CD8 α -CD28+, CD8 α +CD28-, Kul-1+MHCII-, Kul-1+MHCII+, Kul-1-MHCII+ and Bu-1a+ did not present significant differences between all treatments. As expected, these results showed an increased population of naïve and activated T helper (CD4+TCR $\nu\beta$ 1- and CD4+TCR $\nu\beta$ 1+) cells and naïve or memory T cytotoxic (CD8 α +CD28+) cells in blood of unvaccinated and IBV challenged birds, whereas the immune cells dynamics in the vaccinated/challenged group compared to the negative control group (not unvaccinated/not challenged) showed no significant differences, suggesting immune protection against challenge conferred by vaccination, and a systemic exacerbated response mediated by both naïve and activated T helper cells and naïve T cytotoxic cells.