Standardization of immunohistochemistry for detection of avian Infectious Bronchitis Virus - Jaenisch F.R.F.[1], Morés M.A.Z.[1], Esteves P.A.[1*], Trevisol I.M.[1], Silva V.S.[1], Okino C.H.[1], Klein T.A.P.[1], Silva A.D.[2], Ritterbusch G.A.[2], Brentano L.[1]

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Caused by a coronavirus (Infectious Bronchitis Virus - IBV), infectious bronchitis is an important poultry disease for which the development of new diagnostic alternatives is an important issue. Immunohistochemistry (IHC) is a complementary assay for IBV detection once the IHC allows the direct morphologic localization of an infectious agent and the correlation of such agent with corresponding pathologic cellular changes or lesion development. Thus, in the present study, the standardization of an immunohistochemistry assay (IHC) to detect IBV was performed. For this purpose, embryonated SPF eggs were inoculated through allantoic route with 10^3EID_{50}/0,2mL of an IBV strain M41. The embryos were harvested on the third day after inoculation and fixed in 10% formalin for 24 hours and embedded in paraffin. The best results were obtained with the following procedures: The antigen retrieval was performed with 0.05% protease enzyme for 5 minutes. The blocking of endogenous peroxidase activity was carried out with 3% H_2O_2 for 10 minutes. As primary antibody was used the monoclonal anti-protein N-IBV (mouse monoclonal antibody - Prionics) diluted 1:100. Slides were incubated overnight at 37°C . To detection it was used the reaction system LSAB-HRP (DAKO - Carpinteria, USA) and the chromogen 3-amino-9-ethyl-carbazole (AEC). The slides were stained with Mayer's hematoxylin and visualized under light microscope. By the IHC here standardized it was possible to detect the IBV-N protein in smooth muscle cells of the lung vascular wall. Further studies aiming to improve this IBV/IHC technique are in progress.

Key-words: immunohistochemistry, IBV, embryos

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