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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STANDARDIZATION OF IMMUNOHISTOCHEMISTRY FOR DETECTION OF AVIAN INFECTIOUS BRONCHITIS VIRUS

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INTRODUCTION

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.

MATERIALS & METHODS

Embryonated Specific Pathogen Free (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 immunohistochemistry test was evaluated with the following procedures: To the antigen retrieval the 0.05% protease enzyme for 5 minutes method was compared with the heating in a microwave oven at 700W for 10 min and then at 200w for 30 min. The blocking of endogenous peroxidase activity was carried out with 3% H2O2 for 10 minutes. As primary antibody the monoclonal anti-protein N-IBV (mouse monoclonal antibody - Prionics) was tested diluted 1:50, 1:100 and 1:250. Slides were incubated overnight at 37°C and 4°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.

RESULTS & DISCUSSION

The best results were obtained with the following procedures: The antigen retrieval with 0.05% protease enzyme for 5 minutes. The 1:100 dilution of the primary antibody and the slides overnight at 37°C incubation. By the IHC here standardized it was possible to detect the IBV-N protein in smooth muscle cells of the lung vascular wall (**Figure 1**). Further studies aiming to improve this IBV/IHC technique are in progress.

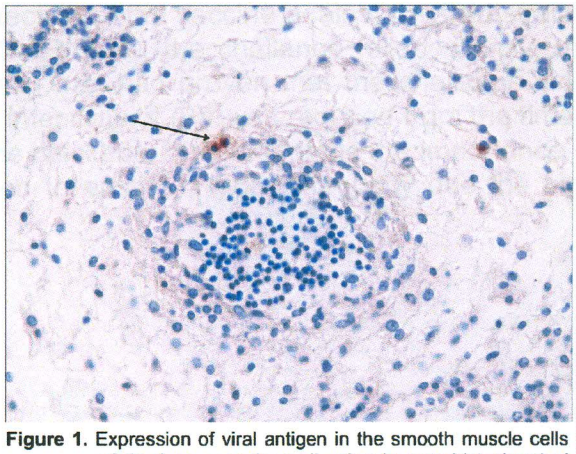

Figure 1. Expression of viral antigen in the smooth muscle cells of the lung vascular wall using Immunohistochemical detection of IBV antigens in chicken embryos after experimental infection with the strain of IBV IHC. 400x

CONCLUSION

Preliminary results of the standardization of immunohistochemistry for the diagnosis of avian infectious bronchitis enabled visualization of positively stained cells in lung tissue of embryos. However, procedures to improve this IBV/IHC technique are in progress.

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