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Immunohistochemistry and RT-PCR for Pathogenesis of Newcastle disease in Chickens

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Introduction

The present experiment was carried out to study the pathogenesis of Newcastle disease by immunohisthochemistry and RT-PCR

Materials and Methods.

Chickens and virus

Two weeks aged specific pathogen-free chickens (White Leghorn) were inoculated with Newcastle disease virus(Kyojeongwon strain : NDV) intranasally. The organ of chickens were necropsied and samples were collected at intervals of 12 hours.

Histopathology and Immunohistochemistry

The tissue samples were fixed in 10% neutral buffered formalin. Thin sections of paraffin-embedded samples were stained by hematoxylin and eosin for histopathology

The avidin-biotin(AB) technique was used for the detection of NDV in paraffin-embeded sections. Anti-NDV monoclonal antibody (Jeno Biotech. 2002) was used as primary antibody.

RT-PCR

Primers were selected in fusion protein cleavage site and collection samples were extracted RNA by RNeasy kit(QIAgen USA). The extracted RNA was performed reverser transcription and polymerase chain reaction.

Results and Discussion

At 48 hour post-inoculation(hpi), clinical findings of the affected chickens were open-mouth breathing, conjunctivitis, watery diarrhea and edema around the eye and neck. At 72 hpi, the chickens showed muscular tremor, paralysis of legs and wings, and coma.

Histopathological results were multi-focal necrosis with hemorrhages in lymphoid aggregates of the intestinal tracts, necrosis of the lympoid tissues, neuronal degeneration and necrosis, and perivascular cuffing. The present study's clinical findings and histopathological results were similarly others results [1, 2]. Immunohistochemically, NDV antigens were detected in the spleen, cecal tonsil, thymus, trachea and lung at 12 hpi. The viral antigens were localized mainly in the cytoplasm of lymphocytes and macrophages, and the results were similar to the previous one [3, 4].

By using of RT-PCR, virus genes were detected in the spleen and proventriculus at 48 hpi. and in the brain at 60 hpi.

The viral antigens were detected as early as 12 hpi, and the results suggested that the immunohistochemical method might be a useful tool for early diagnosis of ND.

References

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