Immunohistochemistry detection of Infectious Bronchitis virus in chicken tissues

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Introduction

Infectious bronchitis virus (IBV) is common in chickens worldwide. IBV belongs to group 3 of the genus Coronavirus of the Coronaviridae family. It was first reported in baby chicks by Schalk and Hawn in 1931, in North Dakota, USA. Although it was thought to be primarily a respiratory disease of chick, avian IBV has since been found to be an economically important disease of adult chicken. Reduced in egg production, enteritis and kidney problems have been reported associated with IBV infection. Kidney disease, variously described as nephrosis, nephritis or uremia was first reported in the USA. In Malaysia, local nephropathogenic IBV was first noticed in 1980 and a prototype MH5365/95 was isolated in 1995. In field and experimental infections the disease appears initially as respiratory infection followed by viremia and nephritis. Histological investigations of the development of kidney lesions throughout the course of infections have shown interstitial inflammatory reaction and tubular degeneration. In respiratory tissues there are cilia loss and degenerative changes of the epithelial cells. In this paper, we described the use of immunohistochemistry technique to detect the location of the antigen in the kidney and respiratory tissues of chicken infected with MH5365/95.

Materials and Methods

Thirty 3-weeks-old SPF chickens were infected with 0.2ml inoculum containing 10^5EID_{50} of virus intra-tracheally. Seven chickens served as control and received 0.2 ml PBS intra-tracheally. About 3-4 chickens and 2 chickens from the infected and control groups, respectively, were killed at 3,6,9,15,18,21 and 24 days post-infection. Upon necropsy, the respiratory, kidney and intestine tissues were collected and fixed in modified Bouin's solution and processed for standard paraffin tissue. Rabbit polyclonal antibody against MH5365/95 at the dilution of 1/50 was used as primary antibody. Immunohistochemical staining was performed according to the manufacturer instruction (Zymed Laboratories, South Sanfrancisco, CA).

Results and Discussion

The control chicken showed negative antigen signal in the trachea, lung, kidney and intestine. In the infected chickens, viral antigens were detected in the tracheal epithelium from day 3 to day 18. Signal was strong and distributed in mucosal epithelium of trachea and in the alveolar mucous gland and submucosa. At day 6, viral antigen was found strongly in the hyperplastic epithelial cells with strong reaction localized in the cytoplasm. In lung, strong positive antigens were detected in the parabronchus wall, atrium, interlobular septum from day 3 to day 15 and become weak starting from day 18 onwards. In kidney, positive signals were detected in the PCT (proximal convoluted tubule), DCT (distal convoluted tubule), CT (collecting tubule) and CD (collecting duct) of the medulla region at day 3, and the signals become more intense thereafter. The signals become less by day 15 and remained only a few in the PCT and DCT. Viral antigens were also detected in the renal glomeruli, glomerular tuft, and around Bowman's capsule. The detection of viral antigens in the kidney and lung tissues are consistent with the distribution of histological changes occurred during the course of the infection. In GIT, almost all parts of the intestine showed positive signals but signals are more intense and longer duration in lower part of the intestine namely the duodenum, ileum, cecal tonsils and cloaca..

Conclusions

The study enable us to understand the type of organ where the virus will persist longer. Where most of the signals in the organs are fading away by the 18 day p.i, the ileum still showing the signals at day 21 pi. This indicate that IBV persist longer in the intestine and may suggest that the feces may served as a virus contaminant.

Benefits from the study

Immunoperoxidase study enable to locate the areas in the organ where the virus replicate and persist. The signals of the antigen-antibody complexes were initially stronger and became weaker as the chicken active antibody developed during the course of infection.

Patent(s), if applicable:

Nil

Stage of Commercialization, if applicable:

Nil

Project Publications in Refereed Journals:
Nil

Project Publications in Conference Proceedings

1. Arshad SS and Al-Salihi K. 2002. Immunohistochemistry detection of Infectious bronchitis Virus antigen in chicken respiratory and kidney tissues. In: Proceedings of the 12th Federation of Asian Veterinary Association Congress/14th VAM Congress, 26-28 August 2002, p51

Graduate Research

Name of Graduate	Research Topic	Field of Expertise	Degree Awarded	Graduation Year
Karima Al-Salihi	Pathogenesis of IBV in SPF chicken: clinical sign, gross lesion and immunoperoxidase	Animal bacteriology	Post-doctoral	
Lai Fu Chong	Detection of infectious bronchitis virus antigen in gastrointestinal tract of chicken by immunohistochemical test	Undergraduate project	DVM	2001

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