

## Microscopy analysis of trachea and kidney of chicken infected with nephropathogenic Infectious bronchitis virus MH5365/95

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### Introduction

Avian Infectious Bronchitis virus is common in chickens worldwide. It belongs to group III coronavirus of the genus Coronavirus of the Coronaviridae family. It was first reported in baby chicks by Schalk and Hawn in 1931, in North Dakota. Although it was thought to be primarily a respiratory disease of chick, avian IBV has since found to be an economically important disease of adult chicken. The disease has been associated with reduced in egg production, enteritis and kidney problem. Kidney disease, variously described as nephrosis, nephritis or uremia was first reported in the USA. In Malaysia, local nephropathogenic infectious bronchitis virus (IBV) was first noticed in the field 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. Respiratory disease is characterized clinically by tracheal rales, coughing and sneezing. Lesions described are serous, catarrhal or caseous exudates in the trachea, nasal passages, lungs congestion around the major bronchi, and cloudiness of the air sacs are also described. Microscopically the principle tracheal lesions are heterophil and mononuclear cells infiltration of the mucosa and submucosa, congestion, epithelial hyperplasia, glandular changes and deciliation. Ultrastructural studies by scanning electron microscopy of the trachea experimentally infected by nephropathogenic IBV shown discrete areas of non-ciliated tracheal mucosa and protruded hyperplastic mucosa into tracheal lumen. Pseudo-membrane presumably consisting of inflammatory cells, degenerated cells and mucus were also noticed. The objective of this study is to examine the cytopathological changes occurred in infected trachea and kidney of chicken following infection by IBV strain MH 5365/95 by light microscopy, SEM and TEM analysis.

### Materials and Methods

Thirty-seven specific pathogen-free (SPF) chickens were used at 21-day old. The local strain MH 5365/95 was used. It had been passage six times in embryonated eggs and twice in chickens, before use in this experiment. Chickens were divided into 2 groups (1<sup>st</sup>=30, 2<sup>nd</sup>=7). Chickens in the 1st group were inoculated intratracheally with 0.2ml of viral inocula containing 10<sup>5</sup> EID<sub>50</sub> ml of IBV-MH 5365/95 strain. The 2<sup>nd</sup> group was acting as control (C), received 0.2ml of PBS by intratracheal route. Clinical signs were recorded daily during 24-days course of post infection (p.i). One and 3-4 chickens from control and infected groups respectively were killed at the interval of 3, 6, 9, 12, 15, 18, 21 and 24 days p.i. Necropsy chickens were examined for gross lesion in trachea and kidney. Pieces of tissues were placed into different fixatives for different analysis procedures. *H & E studies:* The tissues were fixed in 10% phosphate buffered formalin and processed for paraffin block using standard procedure before staining with hematoxylin and eosin. *Transmission studies:* Pieces of trachea and kidney were placed in cacodylate-buffered glutaraldehyde 2.5% and cut into 1mm<sup>3</sup> blocks. The tissues were first pre-fixed in glutaraldehyde at 4 °C for 12 hours, post-fixed for 2 hours in osmium tetroxide, and then dehydrate and embedded in Epon. Ultrathin sections were cut and stained with uranyl acetate followed by lead citrate. All specimens were examined with TEM (Hitachi H-7100) operated at 75KV. *Scanning studies:* Pieces of trachea were fixed with 2.5% glutaraldehyde and post-fixed in 1% osmium tetroxide for 2 hours. The samples were washed with 0.1M sodium cacodylate buffer for 3 changes in 10 minutes, dehydrated through a series of acetone and subjected to critical point drying for 30 minutes. The tissues were add onto stubs using double-sided tap and coated with gold palladium in a sputter coater. Sections were viewed under electron microscope (JOEL 6400 SEM) at 15 kv.

### Results and Discussion

*Microscopy of trachea:* Infectious bronchitis virus predominantly replicates in the upper respiratory tract followed by viraemic spread to various organs namely the kidney, reproductive tissue, lymphoid tissues and intestinal tract. Local nephropathogenic MH5365/95 IBV causes respiratory distress, nephritis and enteritis. The respiratory disease is only transient which last for only 2 weeks and then followed by severe kidney problem. The respiratory system of control chickens appeared normal. Their trachea epithelial cells are covered with cilia. However, early changes in the infected tracheas are observed from day 3 p.i. Epithelial cells from infected trachea showed discrete area of cilia loss and being replaced by microvilli. Many degenerated epithelial cells and other tissue debris are found in the tracheal lumen. In TEM studies, infected epithelial cells showed cell swelling due to increased amount of RER and swollen mitochondria. The latter were occasionally seen with increased number of cristae. Cytopathological changes observed in this study at 3 days p.i, is correlated with the tracheal ciliostasis observed in

another study on tracheal organ culture (TOC) at 3 days pi.. *Microscopy of kidney*: The renal epithelial cells of control chicken appeared normal. However, in the IBV-infected chicken, the kidney were enlarged and swollen. Many tubules including the proximal convoluted, distal convoluted and collecting tubule showed degenerative changes of swollen and vacuolation of their epithelium. Under TEM, the early changes in the infected tubular epithelial cell were represented by cell swelling and reduced electron density of the cytoplasmic matrix. Subsequently, there is an increased in the amount of rough endoplasmic reticulum (RER), together with the increased of fine electron-dense material in its dilated cisternae. This organelle has a higher susceptibility to infection by IBV. Free ribosomes and polyribosomes were also increased due to degranulation of severely dilated RER. The replication process of IBV in renal cells is manifested by the presence of large number of virus particles, mainly in the dilated cisternae of RER. Virus particles were found in endocytotic vesicles of the dilated cisternae of the proliferated RER

### Conclusions

The study provided information on structural changes in the ciliated epithelial cells of trachea and kidney during the course of IBV infection. Being a main site for virus multiplication, cytopathological changes in the trachea are observed at 3 days pi. Cytopathological changes observed in this study at 3 days pi, is correlated with the tracheal ciliostasis observed in another study on tracheal organ culture at 3 days pi. In kidney, the virus was found abundantly in the in the dilated cisternae of RER. Virus particles were found in endocytotic vesicles of the dilated cisternae of the proliferated RER. As the chicken is recovering from the respiratory distress, double-membrane autophagic vacuoles are found scattered in the most infected epithelial cells indicating the activity of cellular repair.

### Benefits from the study

The study provided information on cytopathological changes in the trachea and kidney of chicken following infection with local nephropathogenic IBV.

Patent(s), if applicable:

Nil

Stage of Commercialization, if applicable:

Nil

### Project Publications in Refereed Journals

1. Arshad, SS and Al-Salihi. 2003. Ultrastructure pathology of trachea in experimentally infected with Infectious bronchitis Virus MH 5365/95. *Annals of Microscopy*. 3: 43-47.
2. Arshad, SS and Al-Salihi. 2003. Avian infectious Bronchitis Virus : Ultrastructural pathology of trachea and kidney of chicken infected with local IBV strain. *Annals of Microscopy*. 3: 48-50.

### Project Publications in Conference Proceedings

1. Arshad, SS and Al-Salihi. 2002. Ultrastructure pathology of trachea in experimentally infected with Infectious bronchitis Virus MH 5365/95. In *Proceeding of the 11 th Scientific Conference 2002*, Electron Microscopy Society Malaysia. 17-18 Disember 2002; p280-284
2. Arshad, SS and Al-Salihi. 2002. Avian infectious Bronchitis Virus : Ultrastructural pathology of trachea and kidney of chicken infected with local IBV strain. In *Proceeding of the 11 th Scientific Conference 2002*, Electron Microscopy Society Malaysia. 17-18 Disember 2002; p403-405.
3. Arshad, SS and Al-Salihi. 2001. Ultrastructure Change of the Kidney of Chicken Experimentally Infected with Infectious Bronchitis Virus MH-5365/95 Strain. In: *Proceedings of the 10th Scientific Conference Electron Microscopy Society of Malaysia*, Kuala Lumpur, 8-10<sup>th</sup> November 2001; p270-272
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5. Arshad, SS and Yap, ML. 2000. The ultrastructure of local isolates of avian infectious bronchitis virus. In: *Proceeding of the 9<sup>th</sup> Scientific Conference of EMSM 2000*, p 226-227.

### Graduate Research

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Name of Graduate	Research Topic	Field of Expertise	Degree Awarded	Graduation Year
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<b>Karima Al-Salihi</b>	<b>Transmission and Scanning Microscopy of chicken tissues infected with infectious bronchitis virus.</b>	<b>Animal bacteriology</b>	<b>Post-doctoral</b>
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