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Original Research

Ultrastructural Changes Induced by Nephropathogenic Infectious Bronchitis Virus Isolate (IND/AHL/16/01) In Experimentally Infected Chicken

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Abstract

Infectious bronchitis virus isolate (IND/AHL/16/01) was collected from an outbreak in coloured layer pure line (Dehlam red) and molecularly characterized as nephropathogenic strain by S1 genotyping and phylogenetic analysis. The isolate was experimentally inoculated into chicken by intranasal and intravenous routes @ 104.7 EID50 and after 21 days, birds were euthanized and lungs, kidneys were collected in 2.5% glutaraldehyde for studying the ultrastructural pathology by TEM (Transmission Electron Microscopy). Kidney sections revealed loss of tubular topography, dilation of intertubular junctions, loss of tubular epithelium, swollen nucleus with condensed nucleolus, vacuolation of cytoplasm, electron dense material in cytoplasm and swollen mitochondria and Lung sections showed detached pneumocytes, extensive vesiculation of alveolar epithelial cells. Based on the findings, the virus isolate is known to be nephrotropic, nephropathogenic, inducing a variety of ultrastructural changes in kidneys and lungs.

Keywords: Chicken, IBV, Nephropathogenic, TEM

Introduction

Infectious bronchitis is an acute and highly contagious viral disease caused by infectious bronchitis virus (IBV), a single-stranded, positive-sense RNA virus of Coronaviridae family, causing severe economic losses to the farmers involved in the poultry industry (Cavanagh and Gelb, 2008). In the nephritic form of IBV, prominent lesions noticed are paleness and swelling of the kidneys and mesonephric ducts (Kuppuswamy *et al.*, 2019). The nephritic form of IBV mostly occurs in broiler chickens but can affect young growing pullets and even layers (Meulemans and Van den Berg, 1998). Renal damage associated with different nephropathogenic strains is an increasingly important feature of the IBV infection, especially in broilers, with less respiratory signs (Ziegler *et al.*, 2002) and lesions (Glahn *et al.*, 1989), but with high mortality rates (de Wit *et al*., 2011b).

IBV has an enormous potential to change both by spontaneous mutation and by genetic recombination and recently, different IBV variants have emerged causing nephropathogenic and reproductive problems which demands a dramatic change in vaccination programmes (Cavanagh and Gelb, 2008). IBV is worldwide in distribution, and infection is acquired through inhalation or direct contact with infected birds or premises. In India, nephropathic form of IB usually goes un-noticed because it is confused with metabolic visceral gout. Nephropathogenic strains have emerged from mutation of widely used classical, live attenuated IB vaccines which mainly protects birds from respiratory form of the disease and thus causing altered tissue tropism to kidney causing nephritis, nephropathy and gout has been acquired by these novel strains (Jackwood and de Wit, 2013 and Abdel-moneim *et al*., 2009).

Study of pathogenesis and ultrastructural pathology of new prototype IBV variant may help in developing vaccine strain for better protection of commercial birds against the non- classical IB outbreaks. The present results describe the ultrastructural pathology of nephropathogenic isolate of IB in the chicken lung and kidney tissues by TEM.

Materials and Methods

Virus

Infectious bronchitis virus (IBV) isolate (IND/AHL/16/01) isolated from an outbreak of coloured layer pureline at avian health lab, ICAR- Directorate of Poultry Research (ICAR-DPR, Hyderabad) and was used in the study. The isolate was initially passaged in ECE and showed IBV specific embryo curling, dwarfing and haemorrhage. Allantoic fluid, collected from the ECE, showing embryo specific lesions was then confirmed for IBV with specific primers targeting the S1 gene by RT-PCR according to the standard procedure described by Gelb et al., 2005. The full length S1 gene region was amplified (1.6 Kbp), sequenced and analysed with other IBV types for phylogenetic relationship. The present IBV variant isolate clustered with nephropathogenic variants like 4/91 and 793B. The virus titre was determined by titration in ECE and calculated as per the description of Reed and Muench, 1938 to provide $10^{4.7}$ EID_{50} /ml. The allantoic fluid was checked for other viruses and found negative.

Chicks

A total of 150 twenty-day old Vanaraja chicks were obtained from ICAR-DPR hatchery, Rajendranagar, Hyderabad. Chicks were tested negative for IBV by ELISA. They were randomly distributed into four groups, consisting of 36 chicks in each. First two groups of chicks were inoculated with 1mL and 2mL of $10^{4.7}$ EID50 / 1mL of allantoic fluid (AF) through intranasal route (IN) respectively and third group with 1mL of $10^{4.7}$ EID50 / 1mL of allantoic fluid through intravenous (IV) route and fourth group was kept as control and received only virus free AF. All the experiments were approved by Institute Animal Ethics Committee (IAEC) of ICAR-DPR (IAEC/DPR/18/5).

Processing of Samples for TEM

At the end of 21-day experimental trail, all the birds were euthanized by cervical dislocation and Lungs, kidney tissue samples were collected and preserved in 2.5% glutaraldehyde (PBS based EM grade) and processed for transmission electron microscopy (TEM) as per the standard protocol (Lakshman, 2007). TEM was carried out at Ruska labs, Rajendranagar, Hyderabad.

Results and Discussion

The ultrastructure of kidney sections of intranasal 1ml group revealed lesions like loss of architecture, loss of tubular epithelium topography, epithelial cells showed swollen to pyknotic nucleus with mild to moderate margination of chromatin material with condensed nucleolus, disruption of nuclear membrane, loss of demarcation between nuclear membranes with the cytoplasm. Tubular epithelial cells also showed vesiculation of mitochondria (Fig. 1). I/N 2ml group revealed condensed, apoptotic nucleus. Tubular epithelial cell cytoplasm showed granularity and disintegration (Fig. 2). Kidney sections of I/V 1ml group revealed electron dense VLP's in the cytosol (Fig. 3). These findings were similar to the observations made by Chen and Itakura, 1996; Arshad and Al-Salihi, 2003.

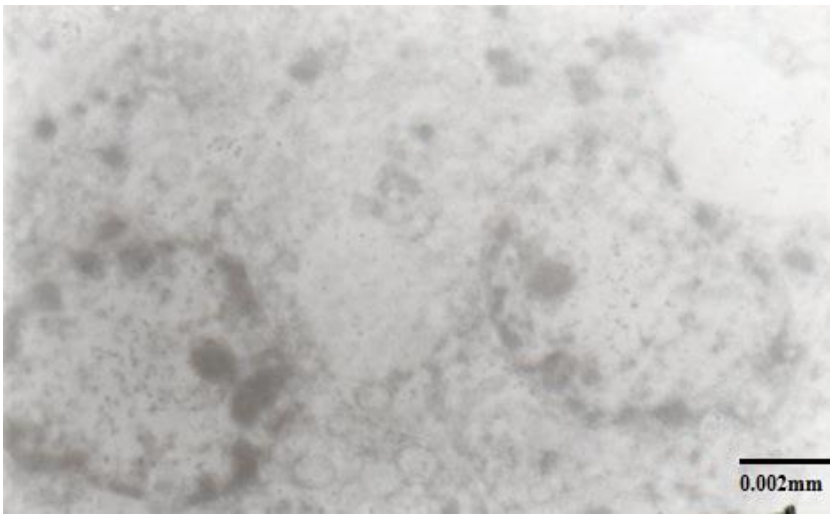

Figure 1: TEM of kidney showing disintegrating nucleus, pyknotic nucleus with large perinuclear vesicle, granular nucleoplasm in I/N 1ml group. UA &LC 13510x

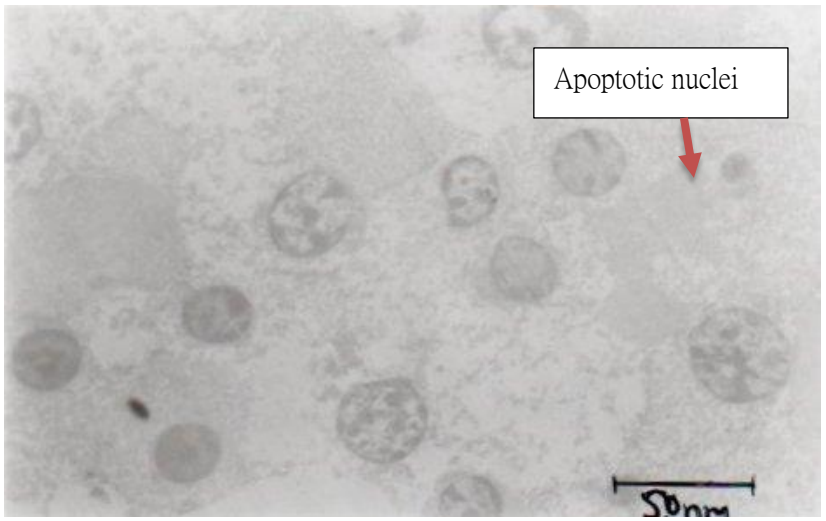

Figure 2: TEM of kidney showing condensed nuclei, apoptotic nuclei, disintegration of tubular epithelial cells, granular cytoplasm and margination of chromatin material in I/N 2ml group. UA &LC 3860x

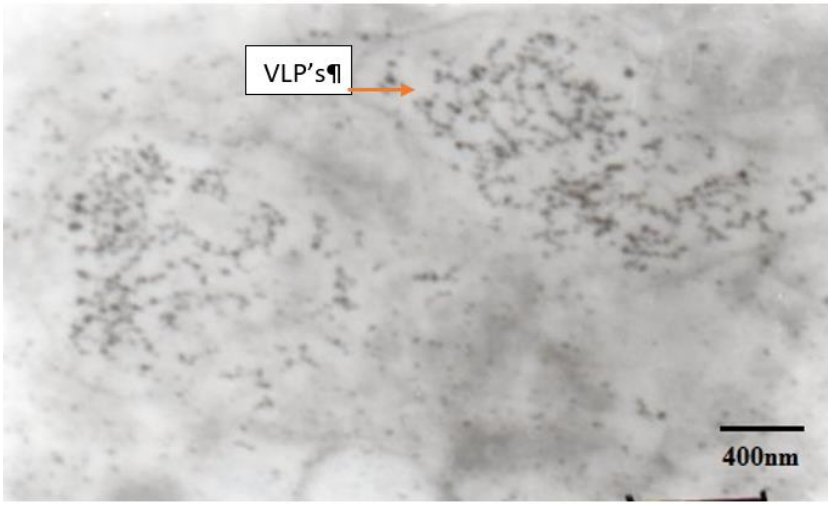

Figure 3: TEM of kidney showing electron dense virus like particles (VLP's) in the cytoplasm of epithelial cells in I/V 1ml group. UA & LC 48250x

The ultrastructure of lung sections of intravenous 1ml group revealed lesions like loss of architecture, detachment of pneumocytes from basement membrane, presence of abnormal, twin nuclei in the existing pneumocytes (Fig. 4). Lung sections of I/N 1ml and 2ml groups revealed similar lesions of extensive vesiculation of alveolar epithelial cells and vesicular cytoplasm respectively (Fig. 5, 6). Few authors (Arshad and Al-Salihi, 2003) also observed similar lesions in lungs of IBV infected chicken with pronounced vesiculation of alveolar epithelium, detachment of pneumocytes from basement membrane.

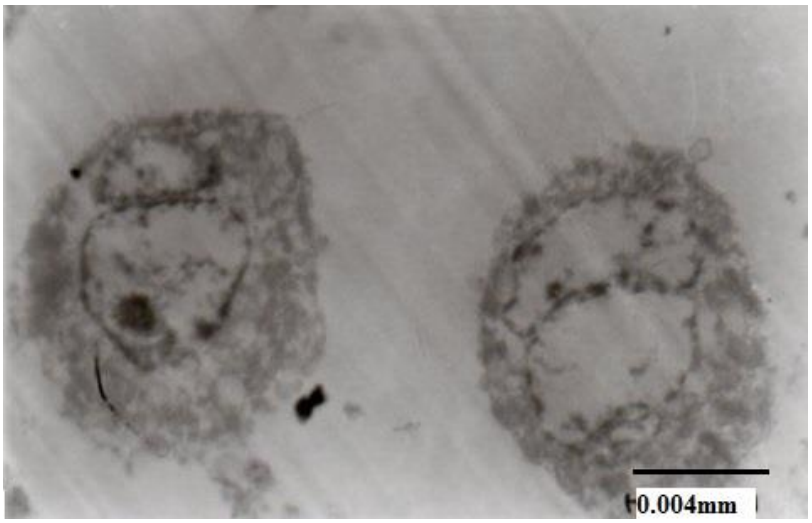

Figure 4: TEM of lungs showing detached pneumocytes and some pneumocytes possess twin nuclei in I/V 1ml group. UA &LC 4825x

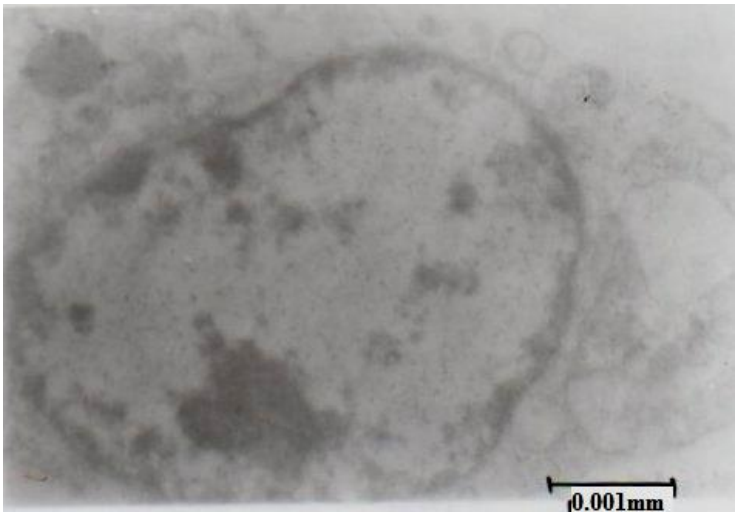

Figure 5: TEM of lungs showing alveolar epithelial cells with extensive vesiculation in I/N 1ml group. UA &LC 9650x

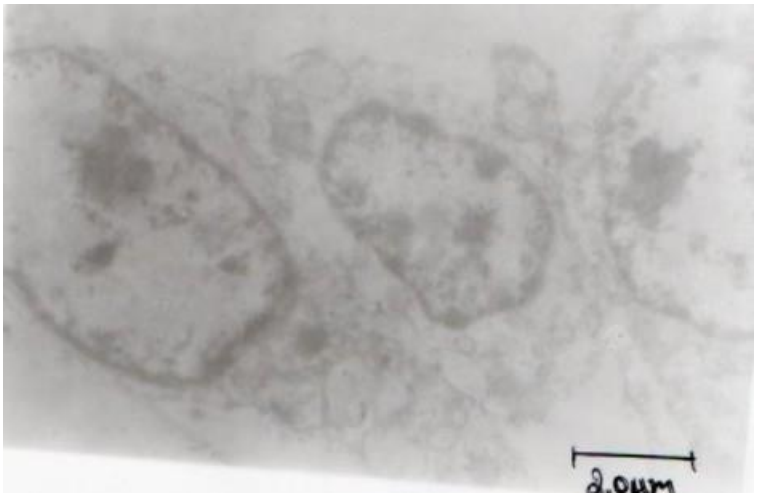

Figure 6: TEM of lungs showing alveolar epithelial cells with extensive vesiculation in I/N 2ml group. UA &LC 9650x

Conclusion

The nephrotropism of the virus isolate IND/AHL/16/01 can be clearly demonstrated from the results, as the virus produced severe ultrastructural changes in the renal tubular epithelium than pulmonary alveolar epithelium, irrespective of the route of inoculation, at a dose of $10^{4.7}$ EID_{50}/ ml of allantoic fluid, and showed lesions varying from swollen to pyknotic nucleus, margination of chromatin, condensation of nucleolus, disruption of nuclear membrane and vesiculation of mitochondria. This confirms that the virus isolate IND/AHL/16/01, is nephropathogenic and hence appropriate precautions must be taken well in advance to prevent the losses in chicken.

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Conflict of Interests

There is no conflict of interest.

Publisher Disclaimer

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