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PATHOLOGY AND MOLECULAR DETECTION OF INFECTIOUS BRONCHITIS VIRUS INFECTION IN BROILER CHICKENS

AMIRA S. SADEK [1]; SARY KH. ABD- ELGHAFFAR [1,2]; KHALED RADAD [1]; KHALED M.A. HASSANEIN [1]; MOHAMMED A. GAMALELDIN [3] AND AHMED K. HASSAN [4]

[1] Department of Pathology and Clinical Pathology, Faculty of Veterinary Medicine, Assiut University, Assiut, Egypt.
[2] Department of Pathology and Clinical Pathology, School of Veterinary Medicine, Badr University in Assiut, Egypt.
[3] Poultry Diseases, Animal Health Research Institute, Agriculture Research Center, Assiut, Egypt.
[4] Department of Avian and Rabbit Medicine, Faculty of Veterinary Medicine, Assiut University, Assiut, Egypt.

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ABSTRACT

Infectious bronchitis is a highly contagious viral disease of chickens, causing significant economic losses in Egyptian chicken farms. In this study, we surveyed the prevalence of infectious bronchitis virus infection in broiler chicken flocks in Assiut Governorate as well as description of its pathological lesions. Pooled samples were collected from 22 broiler chicken flocks suspected to be infected with infectious bronchitis virus. Ten samples were confirmed to be positive for infectious bronchitis virus infection using RT-PCR. Nasal discharge, coughing and gasping were the main signs. Grossly, chickens showed hyperemic trachea with a caseous plug at the bifurcation of the trachea. The kidneys appeared congested and enlarged. Trachea, lungs and kidneys specimens were collected for histopathological examination. Tracheal specimens were also collected for electron microscopy study. Microscopically, the trachea showed complete loss of the epithelial cilia, associated with either necrosis or metaplasia of the lining epithelium. The lungs revealed hemorrhagic pneumonia, necrosis of the bronchial epithelium and thickening in interalveolar tissue with inflammatory edema. The kidneys exhibited swollen glomeruli with hypercellularity, necrosis of renal tubules and severe interstitial hemorrhage. Scanning electron microscopy of the trachea revealed severe deciliation, leaving very short microvilli on the mucosal surface. Transmission electron microscopy demonstrated the presence of viral particles in the epithelial lining. So, despite routine vaccination, IBV is still spreading within broiler flocks in Assiut Governorate, causing severe losses and pathological lesions. This requires further investigation of the immune profiling of these broiler chicken flocks.

Keywords: IBV, Broiler chickens, Histopathology, Electron microscopy, RT-PCR

Corresponding author: Amira S. Sadek
E-mail address: amira.sayed4494@gmail.com
Present address: Department of Pathology and Clinical Pathology, Faculty of Veterinary Medicine, Assiut University, Assiut, Egypt.

INTRODUCTION

Infectious bronchitis (IB) is a highly contagious, acute respiratory viral disease of chickens (Amarasinghe *et al.,* 2017). In North Dakota, USA, IB was first detected in young chicks as a new respiratory disease in the 1930s (Woo *et al.,* 2012; Bande *et al.,* 2016). Infectious bronchitis virus (IBV) is distributed worldwide, with different serotypes and genotypes determined in many countries (De Wit *et al.,* 2011). In Egypt, distinct strains of IBV have been identified since the 1950s (Sheble *et al.,* 1986; Eid, 1998).

IB is characterized mainly by respiratory symptoms such as coughing, difficulty breathing and tracheal rales. Furthermore, it may affect the kidneys causing nephritis (Houta *et al.,* 2021). It also influences the female reproductive system, resulting in reduced production of eggs and low eggs quality (Bhuiyan *et al.,* 2018). The severity of IB increases when secondary infections like mycoplasmosis and colibacillosis after tracheal ciliostasis (Ganapathy & Bradbury, 1999; Hassan *et al.,* 2017).

The disease is caused by IBV a single-stranded RNA virus, one of the genus Gammacoronavirus in the Coronaviridae family (Yuan *et al.,* 2022). The virus genome consists of structural proteins including the spike [S], matrix [M], nucleocapsid (N) and envelope [E] proteins. All these glycoproteins have a major role in the viral replication and occurrence of the disease (de Haan *et al.,* 2000; Bande *et al.,* 2015). The IBV can replicate quickly with high mutations and genomic recombination, resulting in the emergence of various strains (Barjesteh *et al.,* 2020). The IBV affects chickens of all ages, especially broilers with severe signs and a high mortality rate (Abozeid & Naguib, 2020). Transmission of the virus occurs through inhalation or ingestion of viral particles. Furthermore, the virus spread via direct contact with infected chicks or indirect contact with respiratory discharges and fecal droplets (Jackwood & de Wit, 2013).

The virus affects mainly the respiratory system and then disseminates to other organs like the kidneys and reproductive tract. Consequently, the clinical signs and severity of the disease vary depending on the affected organ (Bande *et al.,* 2016). The nephropathogenic variant of the virus causes depression, wet droppings and increased water intake (Cavanagh, 2007). Different strains of IBV induce distinct lesions in many organs according to the pathogenicity of the viral strain, chicken age and the genetic vulnerability of the chickens (Matthijs *et al.,* 2005). IBV is an epitheliotropic virus that multiplies and damages a range of epithelial cells in the trachea, lungs and kidneys (Houta *et al.,* 2021). Controlling the disease is challenging because there is no cross-protection among different genotypes of IBV and the existence of multiple viral genotypes continuously (Thor *et al.,* 2011; Bande *et al.,* 2016). Accordingly, our study aims to survey the prevalence of IBV in broiler chickens from different farms in Assiut Governorate and to detect the pathological findings related to IBV infection.

MATERIALS AND METHODS

1- Sampling

All procedures in our study have been approved by the ethical committee of the Faculty of Veterinary Medicine, Assiut University, Assiut, Egypt, according to the OIE standards for use of animals in research (No.06/2024/0151). Pooled samples were collected from 22 broiler chicken flocks in Assiut Governorates suspected to be infected with IBV. Chickens had previously received IBV vaccines. The examined broiler flocks showed respiratory manifestations such as rales, sneezing, nasal discharge and

coughing. Necropsy was carried out and gross lesions were recorded.

2- Histopathological examination

A- Light microscopy

Tissue specimens from trachea, lungs and kidneys were fixed in 10% neutral buffered formalin for 24 h. Then, tissue specimens were routinely processed for histopathological examination. Briefly, tissue specimens were washed in tap water and immersed in ascending grades of ethyl alcohol (70%, 80%, 90% and 100%) for about a half-hour each for dehydration. Then, tissue specimens were cleared with xylene and embedded in paraffin wax. Tissue specimens were cut into five-micron sections and stained with hematoxylin and eosin stain (Bancroft & Stevens, 1982). Stained tissue sections were examined under a light microscope (CX31, Olympus, Tokyo, Japan) and photographed using a digital camera (Camedia C-5060, Olympus).

B- Electron microscopy

Scanning electron microscopy (SEM)

Tracheal specimens were washed with normal saline and fixed in 5% glutaraldehyde in 0.1 M sodium phosphate buffer for 24 h. Then, specimens were washed with 0.1 M sodium phosphate buffer, dehydrated in ascending series of ethanol concentrations (30%, 50%, 70%, and 90%) for 2 h each, followed by 100% ethanol for 2 days and then by amyl acetate for 2 days. Liquid carbon dioxide was used to apply critical point drying to the specimens. Silver paint was used to stick each specimen to metallic blocks. Specimens were then coated with gold and examined with SEM (JSM 5400 LV, JEOL, Tokyo, Japan) at 15-20 KV in the Electron Microscopy Unit, Assiut University, Assiut, Egypt. Photos were digitally colored with the program Photo Filter 6.3.2.

Transmission electron microscopy (TEM)

Tracheal specimens were fixed in 5% cold glutaraldehyde for 24-48 h. The specimens were washed three to four times with phosphate buffer (pH 7.2) for 20 min each, followed by post-fixation in 1% osmium tetraoxide (O_4S_4) for a period of 2 h. The specimens were then rinsed thoroughly with phosphate buffer at least 4 times. Dehydration was performed in ascending alcohol concentrations (30%, 50%, 70%, 90%, and 100%) for 30 min each (Bozzola & Russell, 1999). Subsequently, the specimens were then embedded in an epon mixture. The embedded blocks were first cut into semi-thin sections about 0.5-1.0 µm thick by a LKB ultramicrotome. The sections were stained with toluidine blue and examined under light microscopy. Selected blocks were sliced into ultra-thin sections (500-700 Å) using a Leica AG ultramicrotome. Then, slices were mounted on copper grids (200 mesh) , contrasted with lead citrate and uranyl acetate, and examined with TEM (100 CXII, JEOL, Tokyo, Japan) operated at 80 KV in the Electron Microscopy Unit, Assiut University, Assiut, Egypt. Photos were taken by a SC30 Olympus camera and digitally colored with the program Photo Filter 6.3.2 to distinguish between different cell and structural types.

3- RT- PCR

Pooled samples from different investigated flocks from trachea and kidneys were washed in sterile saline and then frozen at -80°C. The samples were examined at the Animal Health Research Institute, Dokki, Giza, Egypt. The RNA of the virus was extracted according to QIAamp Viral RNA Mini kit (QIAGEN, catalogue No. 52904) following the company's instructions, the procedure was carried out. The real-time RT-PCR quantification of the virus RNA was done according to QuantiTect probe RT-PCR kit (catalogue No. 204443). The used primers and probe were AIBV-fr (5'-ATGCTCAACCTTGTCCCTAGCA-3'), AIBV-as (5'-TCAAACTGCGGATCATCA CGT-3'), and a probe AIBV-TM (FAM-TTGGAAGTAGAGTGACGCCCAAACTT CA-TAMRA). Primers and probe were synthesized by Metabion (Germany). The

cycling conditions were reverse transcription at 50˚C for 30 min, primary denaturation at 95˚C for 15 min, secondary denaturation at 94˚C for 30 sec, annealing and extension at 60˚C for 45 sec and the number of cycles was 40 cycles. The amplification plot and the threshold cycle were detected using the StepOne software (Applied biosystem) (Meir *et al.*, 2010).

RESULTS

1- Clinical findings

The RT-PCR positive flocks mainly exhibited respiratory manifestations such as coughing, nasal discharge, sneezing, tracheal rales, difficulty breathing and whitish diarrhea. Besides, depression, decreased food intake and ruffled feathers are also noticed.

2- Gross necropsy findings

Most important gross lesions were observed in the trachea and kidneys. The trachea showed hyperemia and presence of a caseous plug at its bifurcation. The caseous plug was also extended into the bronchi and obliterated the bronchial lumen (Fig. 1A). The kidneys revealed swelling, paleness, congestion and petechial hemorrhages on the surface (Fig. 1B).

3- Histopathological findings

A- Light microscopy findings

Trachea of normal chicks showed pseudostratified ciliated columnar epithelium (Fig. 2A). The trachea of infected broiler chickens revealed severe pathological lesions reflect the effect of IBV on the tracheal tissue. The prominent tracheal lesions were necrosis and sloughing of the tracheal epithelium with complete loss of the epithelial cilia. Furthermore, the sub-epithelial tissue including lamina propria and submucosa revealed edema, congestion of blood vessels and mild infiltration with inflammatory cells. There was destruction in the tracheal cartilages accompanied by edema and inflammatory cells reaction in the serosa (Fig. 2B). The most peculiar finding was fibrino-necrotic tracheitis characterized by necrosis of the epithelium with agglutinated blood, presence of fibrin and lymphocytic reaction, leading to thickening in the tracheal mucosa (Fig. 2C). The tracheal lining epithelium revealed metaplasia associated with edema and inflammatory cell reaction in the sub-epithelial tissue (Fig. 2D).

The lungs of a normal chick showed normal alveoli (Fig. 3A). The lungs of infected broiler chickens showed variable pulmonary changes. There were distinct alterations in the bronchi such as necrosis and sloughing of the bronchial epithelium accompanied by congestion of the blood vessels. The interalveolar tissue appeared widened and thickened with inflammatory edema, that was noticed as homogenous faint pink fluid infiltrated with inflammatory cells (Fig. 3B). There was a hemorrhagic pneumonia characterized by necrosis in the alveolar epithelium, hemorrhage into the alveolar lumen with inflammatory cell reaction and thickening in the alveolar wall (Fig. 3C). In addition, some vascular changes such as damage of the endothelial cells and formation of thrombi consisting of fibrin network, RBCs and leucocytes are also observed (Fig. 3D).

Histopathological examination of the renal tissue in a normal chick showed normal renal corpuscle, renal tubules and interstitial tissue (Fig. 4A). Tissue sections from the kidneys of the infected broiler chickens showed various pathological lesions in the glomeruli, tubules and interstitium. Glomeruli showed hypercellularity that manifested by mesangial and endothelial proliferation as well as inflammatory cellular infiltration (Fig. 4B). Renal tubules showed presence of proteinaceous casts that appeared as homogenous pale pink materials in their lumens associated with flattened renal epithelial lining. Also, coagulative necrosis of renal tubular epithelium is expressed by sloughing of the epithelium

and pyknosis of the nuclei associated with interstitial infiltration of inflammatory cells (Fig. 4C). Regarding the interstitial lesions, there was interstitial hemorrhage accompanied by coagulative necrosis of renal tubular epithelium (Fig. 4D).

B- Electron microscopy

Scanning electron microscopy

The tracheal mucosa of normal chicks showed normal epithelial cells covered with abundant cilia (Fig. 5A). On the other hand, the trachea of infected chicks revealed various changes such as complete loss of the epithelial cilia leaving very short microvilli on the mucosal surface and the presence of caseated material on the tracheal mucosa. Furthermore, degeneration of goblet cells, erosions in the tracheal epithelium and the presence of leucocytes (Fig. 5B).

Transmission electron microscopy

The trachea of normal chicks showed normal tracheal mucosa with ciliated epithelial cells (Fig. 5C). However, the trachea of infected chicks revealed the presence of the virus particles in cytoplasmic vesicles within the cytoplasm of the tracheal epithelial cells. The virus particles appeared round to pleomorphic in shape and their surface with club-like projections (Fig. 5D).

4- Molecular detection results

Ten out of 22 examined samples were confirmed positive for IBV using RT-PCR. The results of RT-PCR for positive samples and their threshold cycles are shown in Fig. (6) and are summarized in Table. (1).

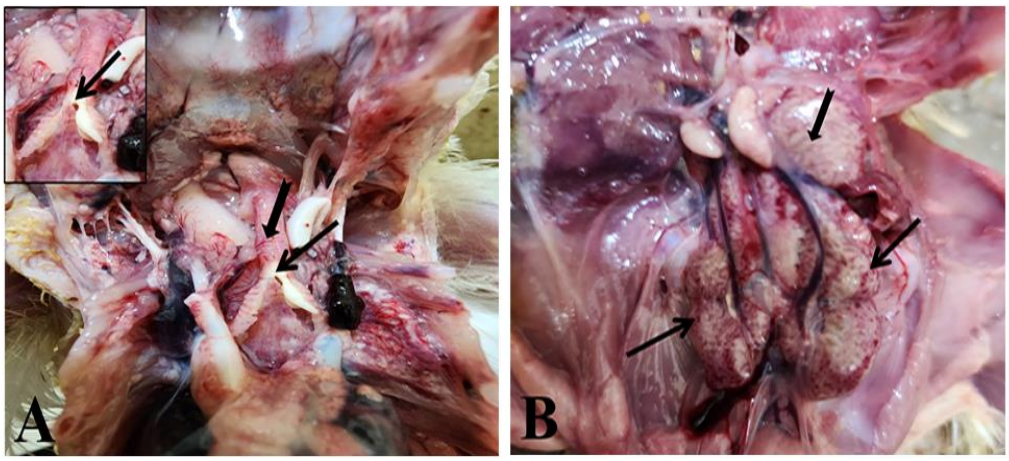

Fig. 1: Representative graphs for gross pathology of IBV on the trachea and kidneys of broiler chickens. **(A)** A trachea of an infected chick showing hyperemia (notched arrow) and presence of a caseous plug at its bifurcation (arrow). **(B)** A kidney of an infected chick showing swelling (notched arrow), congestion and petechial hemorrhages on the surface (arrow).

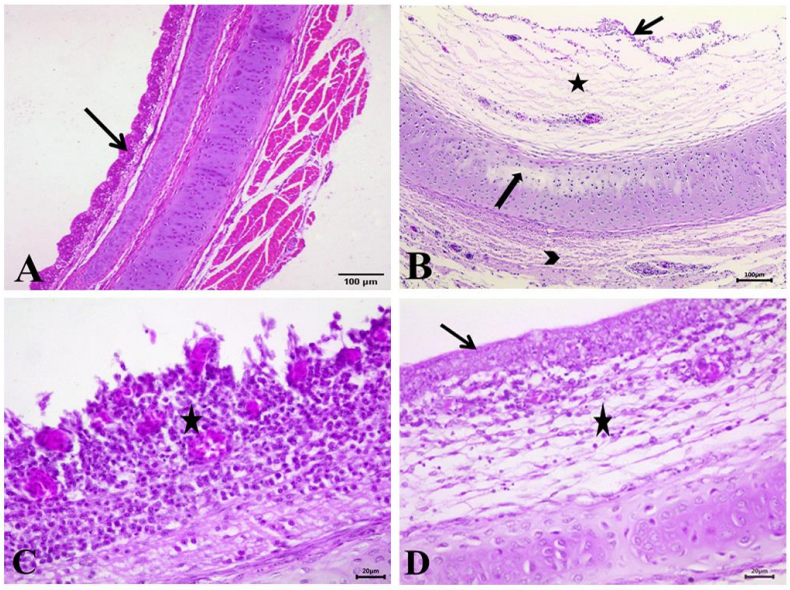

Fig. 2. Representative micrographs for histopathology of IBV on trachea. **(A)** A trachea of a normal chick showing normal pseudostratified ciliated columnar epithelium (arrow). **(B)** A trachea of an infected chick showing necrosis and sloughing of the tracheal epithelium (arrow), edema in sub-epithelial tissue (star), destruction in the tracheal cartilage (notched arrow) and edema in the serosa (arrow head). **(C)** A trachea of an infected chick showing fibrino-necrotic tracheitis characterized by necrosis of the epithelium with agglutinated blood, presence of fibrin and lymphocytic reaction (star). **(D)** A trachea of an infected chick showing metaplasia of the tracheal lining epithelium (arrow), edema and inflammatory cell reaction in the sub-epithelial tissue (star). (H&E).

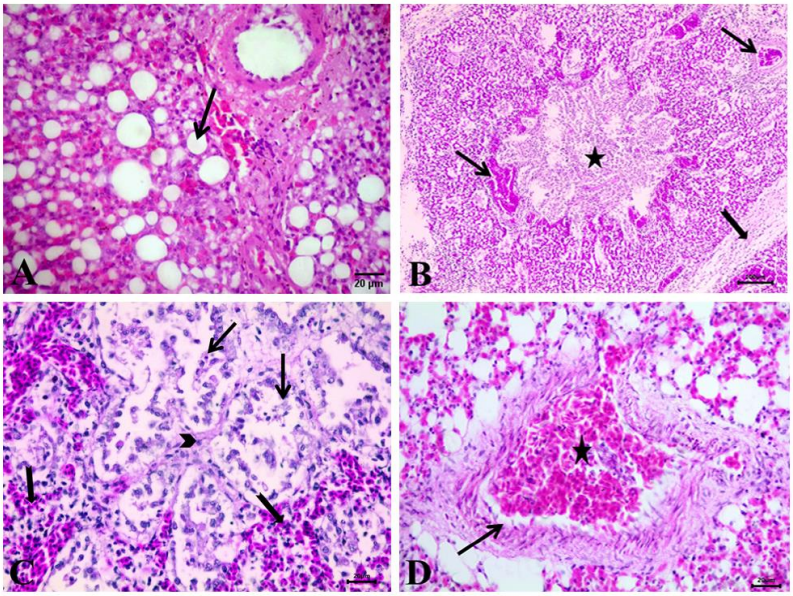

Fig. 3. Representative micrographs for histopathology of IBV on lungs. **(A)** A lung of a normal chick showing normal alveoli (arrow). **(B)** A lung of an infected chick showing sloughing of the bronchial epithelium (star), congestion of the blood vessels (arrow) and thickening in interalveolar tissue with inflammatory edema (notched arrow). **(C)** A lung of an infected chick showing hemorrhagic pneumonia characterized by necrosis in alveolar epithelium (arrow), hemorrhage in alveolar lumen with inflammatory cell reaction (notched arrow) and thickening in alveolar wall (arrow head). **(D)** A lung of an infected chick showing thrombus in the blood vessel (star) and damage of the endothelial cells (arrow). (H&E).

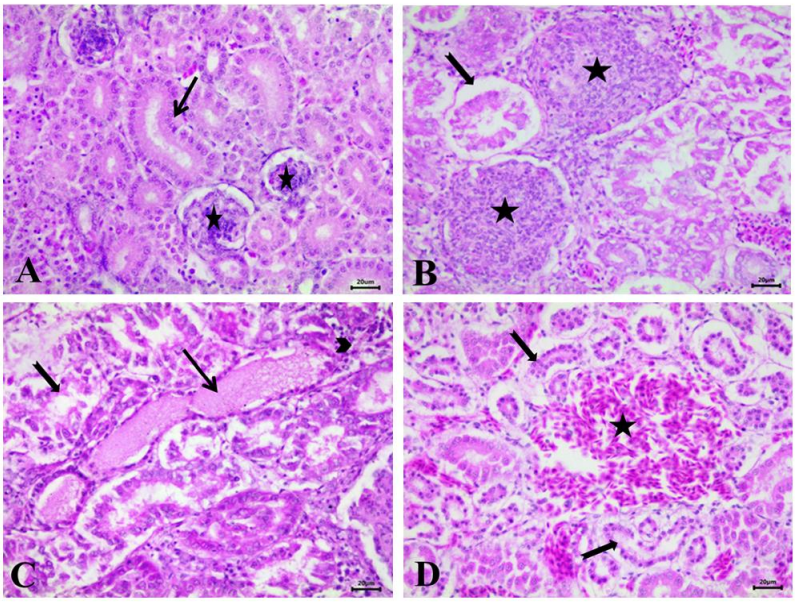

Fig. 4. Representative micrographs for histopathology of IBV on kidneys. **(A)** A kidney of a normal chick showing normal glomeruli (star) and renal tubules (arrow). **(B)** A kidney of an infected chick showing hypercellularity of some glomeruli (star), peritubular edema and coagulative necrosis of the renal tubules (notched arrow). **(C)** A kidney of an infected chick showing proteinaceous cast in the renal tubules (arrow), coagulative necrosis of renal tubular epithelium (notched arrow) and interstitial infiltration of inflammatory cells (arrow head). **(D)** A kidney of an infected chick showing interstitial hemorrhage (star) and coagulative necrosis of renal tubules (notched arrow). **(**H&E).

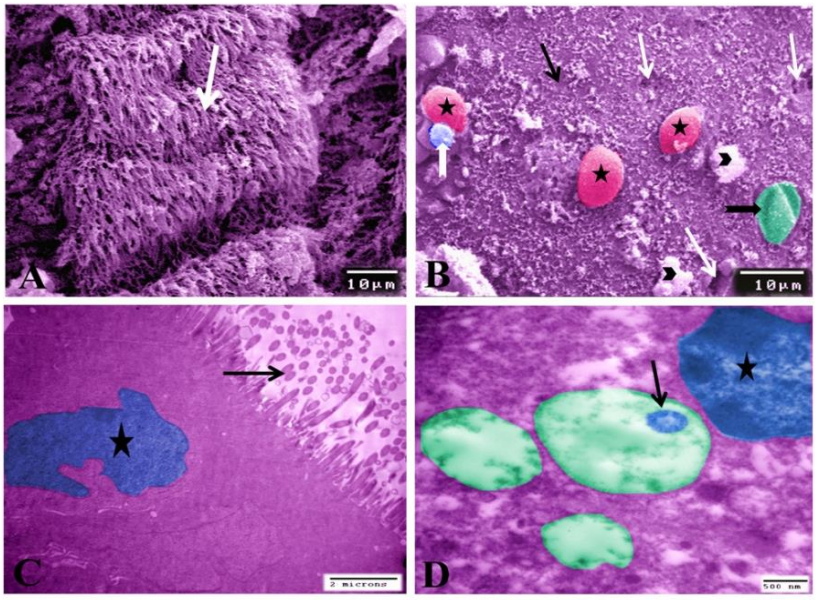

Fig. 5. Digitally colored electron micrographs of the trachea. **(A)** SEM of a trachea from a normal chick showing tracheal mucosa with abundant cilia (white arrow). **(B)** SEM of a trachea from viral infected chick showing complete loss of the epithelial cilia leaving very short microvilli on the mucosal surface (black arrow), presence of caseated material (arrow head), degeneration of goblet cell (black notched arrow), erosions in the tracheal epithelium (white arrow), presence of leucocytes (white notched arrow) and goblet cell (star). **(C)** TEM of a trachea from a normal chick showing normal tracheal mucosa with ciliated epithelial cells (arrow) and normal nucleus (star). **(D)** TEM of a trachea from viral infected chick showing viral particles within cytoplasmic vesicles in the tracheal epithelial cells (arrow) and nucleus (star).

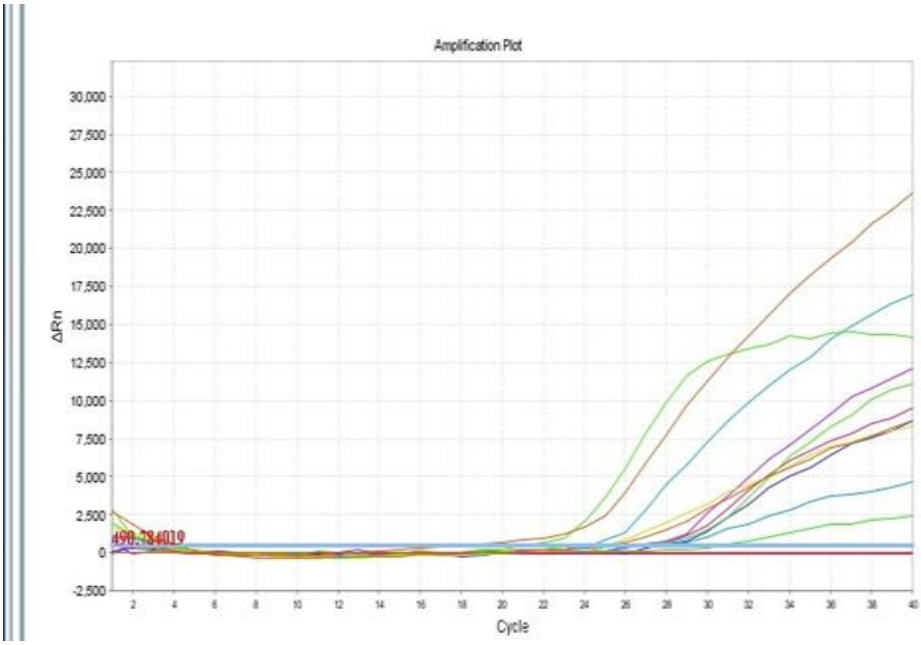

Fig. 6. Amplification plot of RT-PCR for IBV positive samples.

Table. 1. Results of RT-PCR for IBV positive samples and threshold cycle values for each positive sample

Sample No.	Result	Threshold cycle	Sample No.	Result	Threshold cycle
1	+	24	12	-	-
2	-	-	13	-	-
3	+	29	14	-	-
4	-	-	15	-	-
5	+	24	16	-	-
6	+	28	17	-	-
7	+	27	18	-	-
8	+	27	19	+	31
9	+	25	20	-	-
10	+	24	21	+	28
11	-	-	22	-	-

DISCUSSION

In our study, we first investigated the prevalence of IBV infection in broiler chickens in Assiut Governorate by molecular detection. Moreover, gross, histopathological and ultrastructural changes in infected broilers were recorded. Similar to Gola *et al.* (2017), clinical signs in broiler chickens that were positive to IBV in our study were coughing, nasal discharge, tracheal rales and sneezing. Also, Khataby *et al.* (2016) observed similar clinical signs in experimentally infected broiler chickens with IBV strain. On the other hand, Wang & Hou (2023) found that infection of 28-day-old broilers with CK/CH/ GX/202109 IBV isolate did not show any of these clinical signs.

Gross lesions in our study were mainly observed in the trachea and kidneys. The

trachea showed hyperemia and the presence of a caseous plug at its bifurcation. In parallel, Mahmoud *et al.* (2019) investigated the role of IBV in the occurrence of respiratory and renal affections in broiler chicken farms in Egypt. Kamel *et al.* (2010) observed congestion and a caseous exudate in the tracheal bifurcation in broiler farms infected with IB. The mucus exudate that accumulated in the trachea of infected chickens impairs the ciliary action (Arshad *et al.,* 2003). Inconsistency, Grgić *et al.* (2008) reported no tracheal and kidneys gross lesions in experimentally IBV-infected chickens and Najimudeen *et al.* (2022) showed no gross lesions in chickens infected with the Canadian 4/91 IBV isolate.

Gross examination of the kidneys showed swelling, paleness and petechial hemorrhages. Benyeda *et al.* (2010) and Boroomand *et al.* (2012) demonstrated similar lesions in the kidneys of experimentally infected broilers. Enlargement of the kidneys in IBV-infected chickens might be attributed to accumulation of uric acid crystals (Aljubori & Jumma, 2024). On the contrary, Khataby *et al.* (2016) observed no gross lesions in the kidneys of all inoculated broiler chickens with Italy 02 IBV genotype.

Histopathological findings were found in the trachea, lungs and kidneys. The tracheal alterations included sloughing of the epithelium with complete loss of the cilia, inflammatory edema in the sub-epithelial tissue, epithelial metaplasia and fibrino-necrotic tracheitis. These findings were similar to that reported by Mahdavi *et al.* (2007), Benyeda *et al.* (2010), Abou El-Fetouh *et al.* (2016), Hasan *et al.* (2020) who observed desquamation of the epithelial cells, hyperplasia of the epithelium and inflammatory cell infiltration in the sub-epithelial layer in broiler chickens infected with IBV. Also, experimentally-infected chickens with IBV showed similar pathological lesions (Okino *et al.,* 2017).

As IBV is first replicating in the upper respiratory tract, mainly in the trachea (Villarreal *et al.,* 2010), it resulted in degeneration, necrosis and apoptosis (Ignjatovic *et al.,* 2002; Lee *et al.,* 2004; Han *et al.,* 2017). However, Abbood & Ali (2022) showed normal tracheal tissue accompanied with little to no microscopical lesions in some samples from farms positive for IBV. The absence of histopathological lesions may be due to the time of sampling, no lesions can be observed during the three days post-infection (Chousalkar *et al.,* 2007).

Pulmonary changes were in the form of hemorrhagic pneumonia, peribronchial lymphoid cell reaction, necrosis of the bronchial epithelium and thickening of the interalveolar tissue. These results were similarly reported by some authors. For instance, Abou El-Fetouh *et al.* (2016) found thickening of the interalveolar tissue, hemorrhage and desquamation of the bronchial epithelium in naturally infected broiler chicken flocks. Lisowska *et al.* (2021) observed congestion, hemorrhage into the parabronchi lumen and infiltration with inflammatory cells in SPF chicks experimentally infected with IBV GI-23 strain. Najimudeen *et al.* (2022) mentioned hyperplasia of the epithelium of secondary bronchi, proliferation of lymphoid nodules in the lamina propria and hemorrhages inside the parabronchial lumen in experimentally infected chickens with Canadian 4/91IBV.

In our study, kidneys of infected broiler chickens showed hypercellularity of some glomeruli, proteinaceous cast in the renal tubules, coagulative necrosis of the tubular epithelium, interstitial hemorrhage and interstitial nephritis. These renal findings were consistent with Kannaki *et al.* (2021) who reported that all experimentally infected chickens exhibited hypercellularity of glomeruli, necrosis of tubules, interstitial nephritis, and intertubular hemorrhages. Also, Zanaty *et al.* (2016) found similar

results in chickens infected with the IBV Egy/Var-II variant, including glomerular hypercellularity, hemorrhages, renal tubular degeneration and lymphocytic infiltration. Recently, Yan *et al.* (2023) indicated that chickens inoculated with different strains of IBV exhibited severe intertubular lymphocytic infiltration and necrosis of the renal tubular epithelium. Strong inflammatory reactions triggered by cytokines might mediate renal tissue damage after IBV infection (Jang *et al.*, 2013).

Using scanning electron microscopy, the trachea of infected chickens revealed complete loss of the epithelial cilia, erosion in the epithelium, and presence of caseated material on the mucosa. Terregino *et al.* (2008) reported comparable ultrastructural changes in the trachea of non-vaccinated chickens challenged with IB QX such as total mucosal destruction and erosion in the tracheal epithelium.

IBV primarily affects the ciliated and mucus-secreting cells in the upper respiratory tract leading to severe loss of the epithelial cilia and damage of the tracheal mucosa (Seifi & Boroomand, 2015).

Examination of tracheal specimens with transmission electron microscopy revealed the presence of virus particles within cytoplasmic vesicles in the epithelial cells. In parallel, Seifi & Boroomand (2015) detected viral particles within cytoplasmic vesicles in the epithelial cells of chickens experimentally infected with IBV. The virus particles appeared round to pleomorphic in shape with club-like projections on their surfaces. Consistent to our results, Quinteros *et al.* (2022) stated that IBV had a pleomorphic, rounded morphology with surface projections resembling a club. In addition to detection of viral particles, TEM revealed deciliation, increasing in RER and swollen mitochondria. Arshad *et al.* (2003) observed similar findings in the trachea of chickens infected with MH5365/95 IBV.

Molecular detection of the investigated flocks in our study revealed 10 flocks were positive and 12 flocks were negative for IBV using RT-PCR. RT-PCR is usually used as a confirmatory tool for IBV infection in chicken farms. In this context, Dhaygude *et al.* (2018), Rohaim *et al.* (2019), Yaba *et al.* (2023) confirmed IBV infections in broiler chickens using RT-PCR.

CONCLUSION

IBV is spreading within broiler flocks in Assiut Governorate causing major economic losses in the broiler farms, despite routine vaccination. In addition, it damages many tissues such as the trachea, lungs and kidneys leading to various pathological alterations. So, this requires further investigation of the immune profiling of these broiler chicken flocks.

REFERENCES

Abbood, S. and Ali, B. (2022): Histopathological Changes in Lungs and Tracheas of Broiler Chickens Infected With Infectious Bronchitis Virus. *Al-Anbar Journal of Veterinary Sciences, 15*(2): 66–72.

Abou El-Fetouh, M.; Mohamed, M.; Refat, N.; Ahmed, M. and El-Zanaty, A.E. (2016): Pathological Studies on Infectious Bronchitis Disease in Chickens. *Zagazig Veterinary Journal, 44*(3): 248–259.

Abozeid, H.H. and Naguib, M.M. (2020): Infectious bronchitis virus in Egypt: Genetic diversity and vaccination strategies. *Veterinary Sciences* (Vol. 7, Issue 4: pp. 1–9).

Aljubori, A.M.H. and Jumma, Q.S. (2024): Diagnosis of Infectious Bronchitis Infection in Broiler Chicken Farms in Salah Al-Din Governorate. *Egyptian Journal of Veterinary Science(Egypt), 55*(6): 1619–1626.

Amarasinghe, A.; Abdul-Cader, M.S.; Nazir, S.; De Silva Senapathi, U.; Van Der

Meer, F.; Cork, S.C.; Gomis, S. and Abdul-Careem, M.F. (2017): Infectious bronchitis corona virus establishes productive infection in avian macrophages interfering with selected antimicrobial functions. *PLoS ONE, 12*(8): 1-21.

Arshad, S.S., Al-Salihi, K.A. and Noordin, M.M. (2003): Ultrastructural pathology of trachea in chicken experimentally infected with infectious bronchitis Virus-MH-5365/95. *ANNALS OF MICROSCOPY* (Vol. 3): 43-47.

Bancroft, J.D. and Stevens, A. (1982): Theory and practice of histological techniques Churchill. *Livingstone, Edinburgh, London and New York. Pp*: 362–370.

Bande, F.; Arshad, S.S.; Hair Bejo, M.; Moeini, H. and Omar, A.R. (2015): Progress and challenges toward the development of vaccines against avian infectious bronchitis. *Journal of Immunology Research* (Vol. 2015): 1-21 . Hindawi Publishing Corporation.

Bande, F.; Arshad, S.S.; Omar, A.R.; Bejo, M.H.; Abubakar, M.S. and Abba, Y. (2016): Pathogenesis and Diagnostic Approaches of Avian Infectious Bronchitis. *Advances in Virology* (Vol. 2016): 1-11.

Barjesteh, N.; O'Dowd, K. and Vahedi, S.M. (2020): Antiviral responses against chicken respiratory infections: Focus on avian influenza virus and infectious bronchitis virus. *Cytokine* (Vol. 127): 1-11.

Benyeda, Z.; Szeredi, L.; Mató, T.; Süveges, T.; Balka, G.; Abonyi-Tóth, Z.; Rusvai, M. and Palya, V. (2010): Comparative Histopathology and Immunohistochemistry of QX-like, Massachusetts and 793/B Serotypes of Infectious Bronchitis Virus Infection in Chickens. *Journal of Comparative Pathology*, *143*(4), 276–283.

Bhuiyan, Z.A.; Giasuddin, M. and Khan, Z.U.M. (2018): Seroprevalence of infectious bronchitis virus in different types of chicken in Bangladesh. *Asian Journal of Medical and Biological Research, 4*(1): 132–136.

Boroomand, Z.; Asasi, K. and Mohammadi, A. (2012): Pathogenesis and tissue distribution of avian infectious bronchitis virus isolate IRFIBV32 (793/B Serotype) in experimentally infected broiler chickens. *Scientific World Journal*, Volum *2012*: 1-6.

Bozzola, J. and Russell, L. (1999): Electron microscopy: Principles and techniques for biologists.

Cavanagh, D. (2007): Coronavirus avian infectious bronchitis virus. In *Veterinary Research* (Vol. 38, Issue 2: pp. 281–297).

Chousalkar, K.K.; Roberts, J.R. and Reece, R. (2007): Comparative histopathology of two serotypes of infectious bronchitis virus (T and N1/88) in laying hens and cockerels. *Poultry Science*, *86*(1): 50–58.

De Haan, C.A.M.; Vennema, H. and Rottier, P.J.M. (2000): Assembly of the Coronavirus Envelope: Homotypic Interactions between the M Proteins. *Journal of Virology*, *74*(11): 4967–4978.

De Wit, J.J.S., Cook, J.K.A. and Van Der Heijden, H.M.J.F. (2011): Infectious bronchitis virus variants: A review of the history, current situation and control measures. *Avian Pathology* (Vol. 40, Issue 3: pp. 223–235). Taylor and Francis Ltd.

Dhaygude, V.; Moregaonkar, S.; Bulbule, N. and Chawak, M. (2018): Infectious bronchitis virus detection and isolation from broiler chicken. *Journal of Animal Research*, *8*(5): 891–898.

Eid, A.M. (1998): Infectious bronchitis virus infection in Egypt. *Proceedings of the International Symposium on infectious bronchitis and pneumovirus infections in Poultry* (pp. 145–156).

Ganapathy, K. and Bradbury, J.M. (1999): Pathogenicity of Mycoplasma imitans in mixed infection with infectious bronchitis virus in chickens. *Avian*

Pathology, *28*(3): 229–237.

Gola, S.; Shukla, S.K.; Shekhar, S. and Kumar, M. (2017): Prevalence serodiagnosis and histopathological changes in field cases of infectious bronchitis in chickens. *Int J Curr Microbiol App Sci*, *6*(8): 1591–1597.

Grgić, H.; Hunter, D.B., Hunton, P. and Nagy, É. (2008): Pathogenicity of infectious bronchitis virus isolates from Ontario chickens. *Canadian Journal of Veterinary Research*, *72*(5): 403–410.

Han, X.; Tian, Y.; Guan, R., Gao, W.; Yang, X., Zhou, L. and Wang, H. (2017): Bronchitis virus infection induces apoptosis during replication in chicken macrophage HD11 cells. *Viruses*, *9*(8): 1-14.

Hasan, I.I.; Rasheed, S.T.; Jasim, N.A. and Shakor, M.K. (2020): Pathological effect of infectious bronchitis disease virus on broiler chicken trachea and kidney tissues. *Veterinary World*, *13*(10): 2203–2208.

Hassan, K.E.; Ali, A.; Shany, S.A.S. and El-Kady, M.F. (2017): Experimental co-infection of infectious bronchitis and low pathogenic avian influenza H9N2 viruses in commercial broiler chickens. *Research in Veterinary Science*, *115*: 356–362.

Houta, M.H.; Hassan, K.E.; El-Sawah, A.A.; Elkady, M.F.; Kilany, W.H.; Ali, A. and Abdel-Moneim, A.S. (2021): The emergence, evolution and spread of infectious bronchitis virus genotype GI-23. *Archives of Virology* (Vol. 166, Issue 1: pp. 9–26). Springer.

Ignjatovic, J.; Ashton, D.F.; Reece, R.; Scott, P. and Hooper, P. (2002): Pathogenicity of Australian strains of avian infectious bronchitis virus. *Journal of Comparative Pathology*, *126*(2–3): 115–123.

Jackwood, M.W. and De Wit, S. (2013): Infectious Bronchitis. *Diseases of Poultry: Thirteenth Edition* (pp. 139–159). Wiley-Blackwell.

Jang, H.; Koo, B.S.; Jeon, E.O.; Lee, H.R., Lee, S.M. and Mo, I.P. (2013): Altered pro-inflammatory cytokine mRNA levels in chickens infected with infectious bronchitis virus. *Poultry Science*, *92*(9): 2290–2298.

Kamel, K.M.; Bassiouni, A.A.; Afify, M.A. and Rabie, N.S. (2010): The prevalence of Infectious Bronchitis (IB) in some chicken farms in Egypt: III. Cross protection of vaccinated chickens versus field IB virus. *Journal of Veterinary Medical Research*, *20*(1): 351–363.

Kannaki, T.R.; Venkatesh Yadav, J.; Priyanka, E. and Lakshman, M. (2021): Pathology, tissue tropism and antibody response of nephropathogenic infectious bronchitis virus (IBV) Indian isolate in experimentally infected chicken. *VirusDisease*, *32*(3): 568–575.

Khataby, K.; Kichou, F.; Loutfi, C. and Ennaji, M.M. (2016): Assessment of pathogenicity and tissue distribution of infectious bronchitis virus strains (Italy 02 genotype) isolated from moroccan broiler chickens. *BMC Veterinary Research*, *12*(1): 1-10.

Lee, C.W.; Brown, C.; Hilt, D.A. and Jackwood, M.W. (2004): Nephropathogenesis of chickens experimentally infected with various strains of infectious bronchitis virus. *Journal of Veterinary Medical Science*, *66*(7): 835–840.

Lisowska, A.; Pikuła, A.; Opolska, J.; Jasik, A.; Kycko, A. and Domańska-Blicharz, K. (2021): Virulence properties of gi-23 infectious bronchitis virus isolated in poland and efficacy of different vaccination strategies. *Pathogens*, *10*(5): 1-12.

Najimudeen, S.; Barboza-Solis, C.; Ali, A.; Buharideen, S. M.M.; Isham, I.; Hassan, M.S.H.; Ojkic, D.; Van Marle, G.; Cork, S.C.; Van Der Meer, F.; Boulianne, M. and Abdul-Careem, M.F. (2022): Pathogenesis and host responses in lungs and kidneys following Canadian 4/91 infectious

bronchitis virus (IBV) infection in chickens. *Virology*, *566*: 75–88.

Mahdavi, S.; Tavasoly, A.; Pourbakhsh, S.A. and Momayez, R. (2007): Experimental histopathologic study of the lesions induced by serotype 793/B (4/91) infectious bronchitis virus. *Archives of Razi*, *62*(1): 15–22.

Mahmoud, A.; Shahin, A. and Eid, A. (2019): The role of infectious bronchitis virus in respiratory and renal problems in broiler chickens. *Zagazig Veterinary Journal*, *47*(1): 32–44.

Matthijs, M.G.R.; Van Eck, J.H.H.; De Wit, J.J.; Bouma, A. and Stegeman, J.A. (2005): Effect of IBV-H120 vaccination in broilers on colibacillosis susceptibility after infection with a virulent Massachusetts-type IBV strain. *Avian Diseases*, *49*(4): 540–545.

Meir, R.; Maharat, O.; Farnushi, Y. and Simanov, L. (2010): Development of a real-time TaqMan® RT-PCR assay for the detection of infectious bronchitis virus in chickens, and comparison of RT-PCR and virus isolation. *Journal of Virological Methods*, *163*(2): 190–194.

Okino, C.H.; Mores, M.A.Z.; Trevisol, I.M.; Coldebella, A.; Montassier, H.J. and Brentano, L. (2017): Early immune responses and development of pathogenesis of avian infectious bronchitis viruses with different virulence profiles. *PLoS ONE*, *12*(2): 1-17.

Quinteros, J.A.; Noormohammadi, A.H.; Lee, S.W.; Browning, G.F. and Diaz-Méndez, A. (2022): Genomics and pathogenesis of the avian coronavirus infectious bronchitis virus. *Australian Veterinary Journal*, *100*(10): 496–512.

Rohaim, M.A.; El Naggar, R.F.; Hamoud, M.M.; Bazid, A.H.I.; Gamal, A.M.; Laban, S.E.; Abdel-Sabour, M.A.; Nasr, S.A.E.; Zaki, M.M.; Shabbir, M.Z.; Zahran, O.K. and Munir, M. (2019): Emergence and genetic analysis of variant pathogenic 4/91 (serotype 793/B) infectious bronchitis virus in Egypt during 2019. *Virus Genes*, *55*(5): 720–725.

Seifi, S. and Boroomand, Z. (2015): Ultrastructural study of the trachea in experimentally infected broilers with IBV serotype 4/91. *Slovenian Veterinary Research*, *52*(1): 15–21.

Sheble, A.; Sabry, M.Z.; Davelaar, F.G.; Burger, A.G.; Khafagy, A.K.; Moustafa, F.; Moustafa, M.M. and Henna, M. (1986): Present status of infectious bronchitis in Egypt. *J Egypt Vet Med Assoc*, *46*: 393–411.

Terregino, C.; Toffan, A.; Serena Beato, M.; De Nardi, R.; Vascellari, M.; Meini, A.; Ortali, G. Mancin, M. and Capua, I. (2008): Pathogenicity of a QX strain of infectious bronchitis virus in specific pathogen free and commercial broiler chickens, and evaluation of protection induced by a vaccination programme based on the Ma5 and 4/91 serotypes. *Avian Pathology*, *37*(5): 487–493.

Thor, S.W.; Hilt, D.A.; Kissinger, J.C.; Paterson, A.H. and Jackwood, M.W. (2011): Recombination in avian gamma-coronavirus infectious bronchitis virus. *Viruses*, *3*(9): 1777–1799.

Villarreal, L.Y.B.; Sandri, T.L.; Souza, S.P.; Richtzenhain, L.J.; De Wit, J.J. and Brandao, P. E. (2010): Molecular epidemiology of avian infectious bronchitis in Brazil from 2007 to 2008 in breeders, broilers, and layers. *Avian Diseases*, *54*(2): 894–898.

Wang, C. and Hou, B. (2023): A pathogenic and recombinant infectious bronchitis virus variant (CK/CH/GX/202109) with multiorgan tropism. *Veterinary Research*, *54*(1): 1-14.

Woo, P.C.Y.; Lau, S.K.P.; Lam, C.S.F.; Lau, C.C.Y.; Tsang, A.K.L.; Lau, J.H.N.; Bai, R.; Teng, J.L.L.; Tsang, C.C.C.; Wang, M.; Zheng, B.-J.; Chan, K.-H. and Yuen, K.-Y. (2012): Discovery of Seven Novel Mammalian and Avian

Coronaviruses in the Genus Deltacoronavirus Supports Bat Coronaviruses as the Gene Source of Alphacoronavirus and Betacoronavirus and Avian Coronaviruses as the Gene Source of Gammacoronavirus and Deltacoronavi. *Journal of Virology*, *86*(7): 3995–4008.

Yaba, Z.H.; Abdulrahman, N.R. and Hassan, S.M. (2023): Molecular and Pathological Investigation of Infectious Bronchitis in Broiler Flocks in Sulaimani Province, Iraq. *Al-Anbar Journal of Veterinary Sciences*, *16*(2): 85–97.

Yan, W.; Yang, Q.; Huang, S.; Liu, S.; Wang, K.; Tang, Y.; Lei, C.; Wang, H. and Yang, X. (2023): Insights on genetic characterization and pathogenesis of a GI-19 (QX-like) infectious bronchitis virus isolated in China. *Poultry Science*, *102*(7): 1-10.

Yuan, S.; Cheng, Q.; Guo, J.; Li, Z.; Yang, J.; Wang, C.; Liang, Z.; Zhang, X.; Yu, H.; Li, Y.; Huang, S. and Wen, F. (2022): Detection and genetic characterization of novel infectious bronchitis viruses from recent outbreaks in broiler and layer chicken flocks in southern China, 2021. *Poultry Science*, *101*(10): 1-8.

Zanaty, A.; Arafa, A.-S.; Hagag, N. and El-Kady, M. (2016): Genotyping and pathotyping of diversified strains of infectious bronchitis viruses circulating in Egypt. *World Journal of Virology*, *5*(3): 125-134.

الفحص الباثولوجي والجزيئي لعدوى فيروس إلتهاب الشعب الهوائية المعدي في دجاج التسمين

اميره سيد صادق ، ساري خليل عبد الغفار ، خالد رداد ، خالد محمد أحمد حسانين، محمد احمد جمال الدين ، احمد خلف حسن

Email: amira.sayed4494@gmail.com Assiut University web-site: www.aun.edu.eg

التهاب الشعب الهوائية المعدي هو مرض فيروسي شديد العدوى يصيب الدجاج، ويسبب خسائر اقتصادية كبيرة في مزارع الدجاج المصرية. تم في هذه الدراسة إجراء مسح مدى إنتشار فيروس إلتهاب الشعب الهوائية المعدي في قطعان دجاج التسمين بمحافظة أسيوط وكذلك وصف آفاته المرضية. تم جمع عينات من ٢٢ قطيع دجاج تسمين يشتبه في إصابته بفيروس إلتهاب الشعب الهوائية المعدي. تم التأكد من أن عشر عينات إيجابية لعدوى فيروس إلتهاب الشعب الهوائية المعدي بإستخدام تفاعل البلمره المتسلسل التوقيتى الحقيقى. وكانت الأعراض الرئيسية هي سيلان الأنف والسعال واللهاث. وبالفحص العينى للدجاج ظهر إحمرار القصبة الهوائية مع وجود إنسداد متجبن فى تشعب القصبة الهوائية. كما أن الكليه ظهرت محتقنة ومتضخمة. تم جمع عينات من القصبة الهوائية والرئه والكليه للفحص النسيجي. كما تم جمع عينات من القصبة الهوائية للفحص بالميكروسكوب الإلكتروني. مجهريًا، أظهرت القصبة الهوائية فقدانًا كاملاً للأهداب الظهارية، مرتبطة إما بنخر أو تحول في الظهارة المبطنة. كما شوهد إلتهاب رئوي نزفي فى الرئه ،ونخر ظهارة الشعب الهوائية ، وسماكة في الأنسجة البينية مع وذمة إلتهابية. أظهرت الكليه كبيبات منتفخة مع تكثر الخلايا، ونخر الأنابيب الكلوية، ونزيف خلالي حاد .أظهر الميكروسكوب الإلكتروني الماسح للقصبة الهوائية فقدانًا كاملاً للأهداب الظهارية ، مما ترك زغيبات صغيرة جدًا على سطح الغشاء المخاطي. كما أوضح الميكروسكوب الإلكتروني النافذ وجود جزيئات فيروسية في خلايا البطانة الظهارية. لذلك، على الرغم من التحصين الروتيني، لا يزال فيروس إلتهاب الشعب الهوائية المعدي ينتشر داخل قطعان دجاج التسمين بمحافظة أسيوط، مما يسبب خسائر فادحة وآفات مرضية. وهذا يتطلب مزيدًا من التوصيف المناعي لقطعان دجاج التسمين.