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Serological study of infectious bronchitis virus in vaccinated broiler chickens

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Abstract:

Gammacoronavirus, which causes avian infectious bronchitis (IB), is characterized by respiratory and urogenital disease and causes substantial financial loss in both layers and broiler chickens.

In order to determine the prevalence of antibodies against the infectious bronchitis virus (IBV) in immunized birds that showed respiratory signs as well as to evaluate the pathological changes in the trachea and lung, this preliminary study was carried out. The current work is designed to evaluate the presence of humoral immunity against IBV among six vaccinated broiler flocks in Nineveh governorate suffering from respiratory signs. At the same time, clinical signs, necropsy lesions, and histopathological findings were studied. Six pooled tracheal (T) samples and six pooled kidney (K) samples from six flocks were collected for rapid testing. On the other hand, 138 serum samples were collected to investigate the level of IBV antibodies using the indirect ELISA® test. The results of the IBV antigen rapid detection test from suspected field samples revealed that 83.3% of tracheal samples were positive, while positive kidney samples showed 66.6%. With respect to the ELISA results, they show the highest titer of 12910 and the lowest titer of 6379, with a titer mean of 9471.5. Considering the overall picture of the health status, all flocks display pathological changes. This study demonstrates that the detection of Ab titers against IBV can be successfully used for the early and rapid screening of IBV in vaccinated or infected flocks. However, due to a lack of vaccine specificity against endemic strains of IBV in Iraq, IBV infection has persisted as a challenge on chicken farms.

Keywords: Infectious bronchitis, Broilers, Rapid flow test, ELISA, Histopathology.

Introduction:

Infectious Bronchitis (IBV) is a highly spreading disease affecting meat and layer chickens, caused by an RNA coronavirus (1,2).The disease was first described in 1931, and now it is spreading all over the world, including Iraq (3, 4). IBV causes a huge impact on poultry production, including body weight reduction and increased mortality, and negatively affects the quantity and quality of egg production.Morbidity of 100% and 84% mortality, especially in chicks 6 weeks old, was registered (5), depending on strain virulence, flock immunity, age, and secondary infections (6, 7).The importance of IBV disease relies on its short incubation period (24–48 h) (8) and the fact that it affects the respiratory, digestive, and urogenital systems (9).Birds may remain after infection in a carrier state (10), with different antigenic, non-cross-protected pathogenic serotypes, and in order to provide reliable evaluation based on the local condition, numerous commercial vaccines have been

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created against IBV protection. (4,11,12,13).An enzyme-linked immunosorbent assay (ELISA) is usually used to assess IBV immunity and the efficacy of vaccination regimens (12, 5, 13). The aim of this study was to ascertain the frequency of IBV-specific antibodies in broiler birds that have received the IBV vaccine in Nineveh governorate.

Material and Methods:

Study design:

Broiler flocks:

The investigation covered six broiler flocks distributed in Nineveh governorate (A-F) during 2020–2021 (Table 1). Commercial birds are raised on cardboard paper or litter from half-opened houses. Commercial feed and water were given ad libitum. At one day of age, flocks were vaccinated with a combined live IBV vaccine (Nobilis® ND Clone 30, Nobilis® IB Massachusetts H120) and killed by a combined AI and ND vaccine. Revaccinations against Newcastle disease (Nobilis®NDV Clone 30) were given at 2nd or 3rd weeks. Infectious bursal disease vaccine (Nobilis®Gumboro E228) was given at 7 or 14 days of age.

Table 1: Broiler flocks, chicken number and age of infection

Farm	Broiler's number	Age (weeks)
A	18500	3-5
B	14000	4-5
C	14200	3-4
D	12900	3-4
E	18500	5
F	12000	4

Rapid detection of IBV antigen:

Rapid IBV. The Ag test kit (Bionote®, Inc., Korea) was used for rapid examination of six pooled tracheal and six kidney samples from six flocks using cotton tips from sick and dead birds for IBV antigen detection as per the manufacturer's recommendations. Swabs were then inserted into a diluent tube and mixed thoroughly for the extraction of the virus. Extracted samples were slowly added into the sample hole using the disposable dropper. After 5–10 minutes, a purple color moved to the center of the cassette window, indicating a positive result.

Collection of blood samples:

Blood samples (no =138) were collected (23 samples per broiler flock) from clinically affected farms for the detection of serum IBV antibodies. Indirect ELISA using IBV pre-coated plates and pre-diluted reagents was used (Biochek, UK). Blood samples were collected by venipuncture (1.0 ml per bird) of the brachial vein using a 3-ml disposable syringe in gel-containing test tubes. The collected blood was centrifuged (Hettich Centrifuge, Germany, EBA-20) for 10 minutes at 3000 rpm. After centrifugation, the sera were frozen at -20 C until processing to assess the presence of the anti-IBV antibody. An Elx800 reader with a 405-nm filter (biotek™) was used to read the titration on microplates. Positive samples were those that had at least one sample positive with an optical density (OD) > 0.2.

Calculation of the S/P ratio:

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The calculation of the S/P ratio and V index was accomplished according to the Biochek kit, UK.

Statistic evaluation:

The chi-square test (2-test) was used to analyze the study data. P values less than 0.05 were regarded as statistically significant in all chi-square tests.

Ethical Consideration:

Ethical review and approval were granted by the Scientific Board of the college of Veterinary Medicine, University of Mosul, Iraq.

Results:

Detection of IBV antigen from suspected field samples:

The results of the rapid IBV Ag test from collected samples are shown (Table 2 and Fig. 1). 12 pooled samples of tracheal and kidney swabs were collected as recommended by Bionote®, Inc., from sick and dead birds. Five pooled tracheal samples (83.3%) and four (66.6%) kidney samples were positive out of six samples examined in each

Table (2): Type of swabs taken from broilers at different ages and locations

Farm	No. of samples (pooled)	Swabs type	positives	Percentage
A	1	T*	+	T (83.3%) K (66.6%)
	1	K**	-	
B	1	T	+	
	1	K	-	
C	1	T	+	
	1	K	+	
D	1	T	+	
	1	K	+	
E	1	T	-	
	1	K	+	
F	1	T	+	
	1	K	+	

T*= Tracheal swab; K*=Kidney swabs.

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Figure (1): showing purple color band at both 'T' and 'C' lines (positive).

Infectious bronchitis virus (IBV) titers detection using the ELISA technique:

Table 3 showed the highest titer of 12910 and the lowest titer of 6379 with a titer mean of 9471.5. All broiler flocks (100%) had high antibody titers. However, flock B recorded the lowest antibody level (6379), with a vaccination index of more than 70.

Table (3): mean antibody titers and vaccination index of broiler chickens in Nineveh governorate

Farm	ELISA Antibody titer Suspected titer of infection	Vaccination index Suspected titer of infection
A	7902	88.61
B	6379	76.91
C	10060	279.76
D	9533	95.74
E	12910	355.76
F	10045	134.86

Clinical signs:

The signs of affected broiler chicks (2–5 weeks of age) vary in severity and include aggregation around heat sources with lacrimal discharges and other respiratory signs, with different morbidities and mortalities. Birds developed airsacculitis, Fibrinous pericarditis, perihepatitis, peritonitis, and air sacculitis (polyserositis).

Gross lesions:

Necropsy of live and dead birds is characterized by tracheitis with tracheal secretions and exudate in the trachea. Formation of mucoid plugs of pus in the lower trachea and in the primary or secondary bronchi of the lungs (Fig. 2.A, B, and C).

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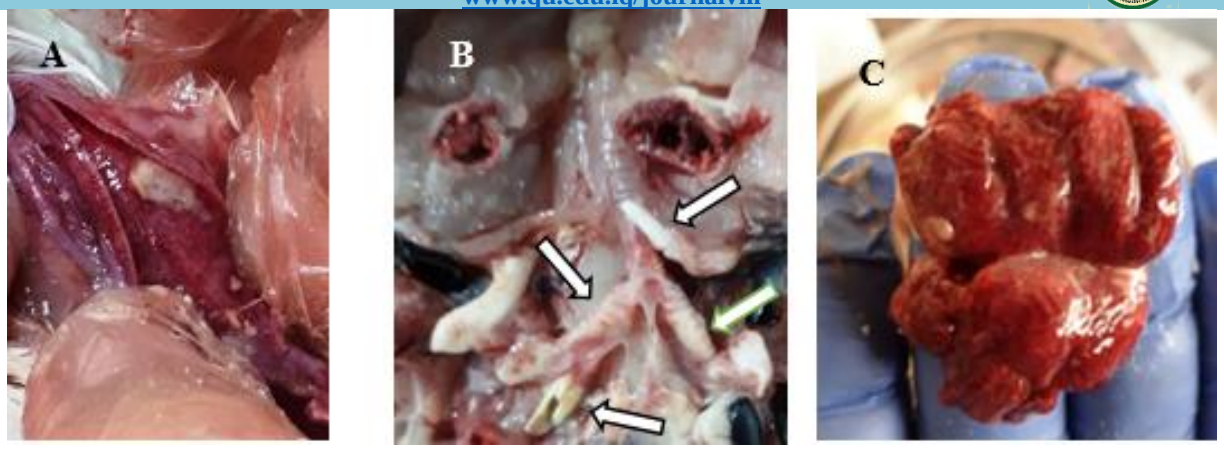
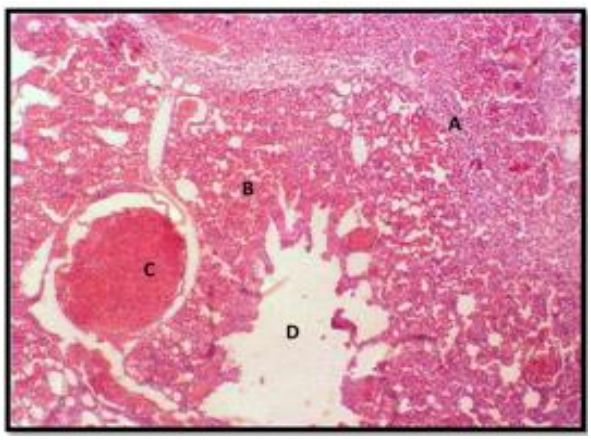


Figure (2): Necropsy findings in broilers suspected to be affected with IBV. **A**: Tracheitis; **B**: Plagues in the main bronchi of the lungs (arrows); **C** Fibrinous pneumonia.

Histopathology:

Histopathological examination of the lungs, tracheas, and bronchi of positive live broilers was shown in figures 3–11. Lungs of affected broilers show pathological changes characterized by bronchopneumonia, hemorrhages of lung parenchyma with congestion of blood vessels, and severe bronchitis with necrosis of bronchioles epithelium accompanied by thickening of the bronchial wall by inflammatory cells. The mucosa of the trachea of chickens is edematous, with massive monocytes and lymphocytes and a loss of mucous glands. Cilia are sloughed mucosal cells with granulocyte infiltration. Hyperplasia and metaplasia are characterized by massive infiltration of the lamina propria by lymphoid cells (Figs. 3–5). Fibrinous caseous exudate was evident in the lumen of the bronchial bifurcation: necrosis, epithelial cell desquamation, hemorrhage, and severe congestion of blood vessels (Figs. 6–10).



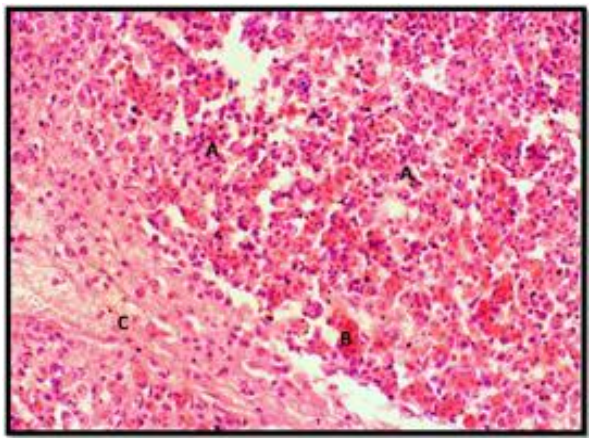


Fig. (3): A photomicrograph of a chicken's lung because of IB infection shows bronchopneumonia characterized by thickening of the bronchiolar wall by inflammatory cells (A), hemorrhage (B), congestion of blood

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vessels (C) and necrosis of epithelium of bronchioles (D).

Fig (4): Lung with IB infection shows severe pneumonia with inflammatory cell infiltration (lymphocytes and polymorphnuclear cells) in alveolar tissue (A), hemorrhage in interstitial tissue (B), increased fibrous connective tissue in between (C).

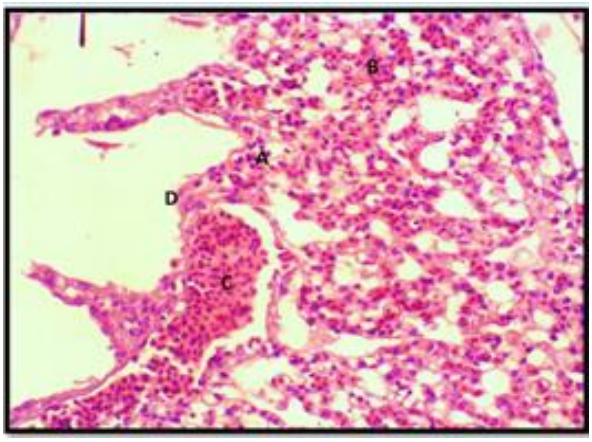

Fig.(5):Lung with IB infection shows bronchopneumonia, thickening of bronchiolar wall by inflammatory cells (A), hemorrhage (B), congestion of blood vessels(C)and necrosis of epithelium of bronchioles(D).H&E stain, 400X.

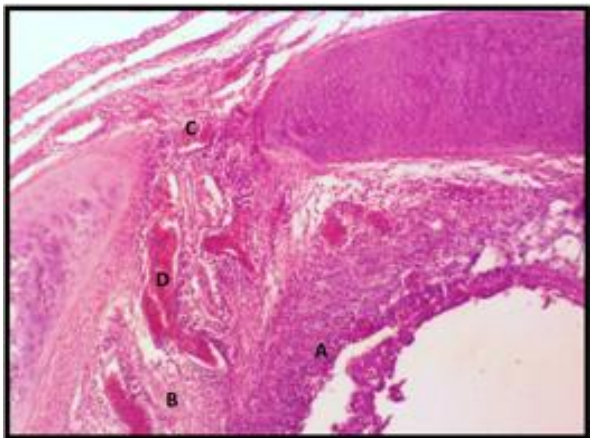

Fig.(6):Trachea with IB infection shows severe bronchitis characterized by thickening of vessel by inflammatory cells (A) and caseous exudate (B), hemorrhage (C) and severe blood vessel congestion (D).

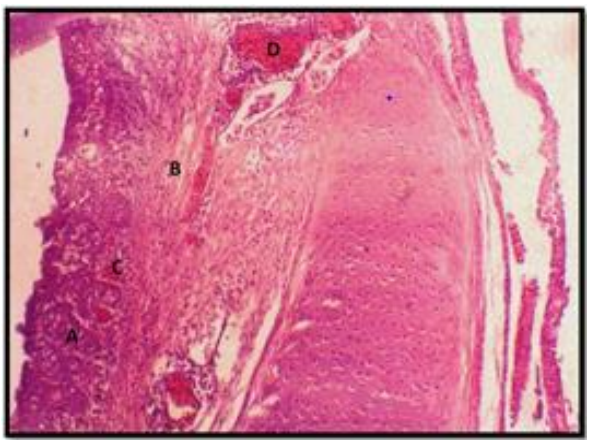

Fig.(7): Trachea with IB infection shows severe bronchitis characterized by thickening of bronchial wall by inflammatory cells (A) and caseous exudate (B), hemorrhage (C) and severe blood vessel congestion (D).

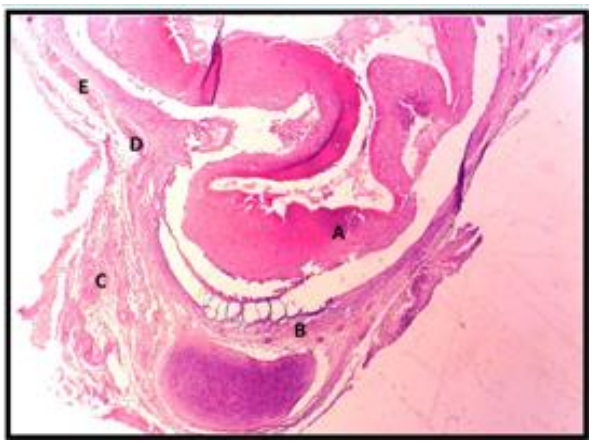

Fig. (8): Bronchial bifurcation with IB infection shows caseous exudative bronchitis (A), different inflammatory cell infiltration (B), hemorrhage (C), hyperemia (D) and blood vessel congestion (E).

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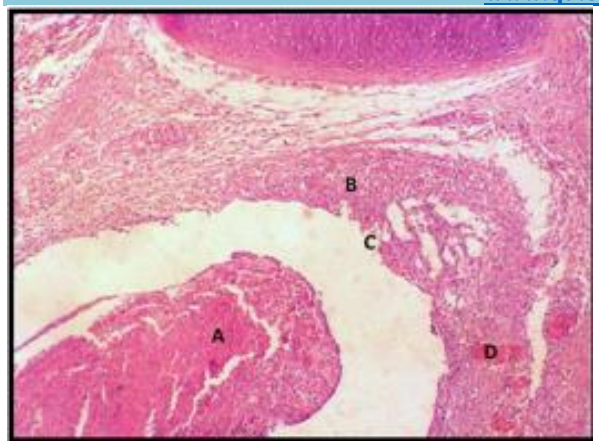

Fig.(9): Bronchial bifurcation with IB infection shows severe bronchitis characterized by the presence of caseous exudative bronchitis in the lumen (A), infiltration of inflammatory cells (B), necrosis and desquamation of epithelial cells (C) and of blood vessel congestion (D).

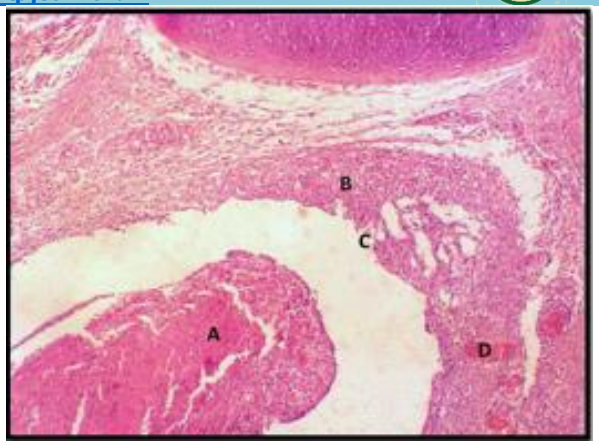

Fig. (10): Bronchial bifurcation with IB infection shows severe bronchitis characterized by presence of caseous exudate in the lumen (A) inflammatory cells infiltration (B), epithelial cell necrosis and desquamation (C) and blood vessel congestion (D).

Discussion:

Infectious bronchitis in chickens has a potential negative impact on poultry production (14). Here in Iraq, infectious bronchitis virus (IBV) was previously reported by (15, 3). The virus is highly contagious and is characterized by rapid airborne transmission, in addition to other natural animate and inanimate routes of infection (16). Gross lesions of the trachea, bronchi, and air sacs encountered at necropsies of affected birds include white caseous plaques of the bifurcation of the bronchi and signs of pneumonia (16). An all-in, all-out system with strict biosecurity and vaccination is highly required to protect chickens against challenges with virus strains of IBV. (9, 17–16), according to the current results and others using the H120 vaccine serotype in Iraq and in Nineveh governorate, it is seemingly not enough to provide protection due to the high mutation and recombination rates of the virus (18, 19). Antigenic variation in IBV vaccines is not well understood due to the variations in virulence and the increasing number of IBV serotypes (6). and because of the lack of a protective vaccine against endemic strains of IBV, continuous identification of the local serotype and the manufacturing of new generations of vaccines are so important for the eradication of IBV. (20) Autogenous serotypes should be strictly used for the development of new vaccines for an effective vaccination program (5).One of the easy sensitive assays, is the rapid detection of IBV. infections using immunochromatographic strip (9), to detect different IBV genotypes and variants (in addition to the other serological methods like HI, AGPT, ELISA and VNT) used for controlling and eradication of IBV. (4,21,22).An enzyme-linked immunosorbent assay (ELISA) is commonly used for the determination of different respiratory disease antibodies (23). Regarding present results, it appeared that IBV suspected to be in the affected flocks had ELISA titers of more than 4000 or 5000 and, more than 70, reflected a titer related to infection status but not a vaccination

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titer (24). Current ELISA results may be supported by Monreal and colleagues, who showed that the quantification of the mean antibody titer in sera of vaccinated chickens against infectious bronchitis virus by ELISA test in broilers vaccinated with live IBV vaccine (4/91) after 3 weeks post infection was 3125.6, and 4154.6 in chicks vaccinated with Ma5 at one day old chicks, and 5048.6 in chicks vaccinated with H120, while the mean when classical and variant IBV vaccines were used was 4739.75 (25). Using the classical vaccine of local broiler flocks, our study indirectly confirmed that local IBV exists as different types that do not have cross-protection, and that is why it is very difficult to control. However, IBV control can be improved by improving management and air quality, decreasing bird density, and employing an all-in/all-out strategy (26).So, to progress vaccine production for emergencies (27, 28–29), novel vaccine technology development must be fast considering mutation or recombination pressure. According to current results, it should focus on the risk factors for IBV vaccine failure, including both different immunization routes and vaccination periods, and also pay attention to the challenge of potential immunosuppression causes in chickens.The clinical signs seen in the investigated broiler farms were also reported by other investigators (5, 30), but with different severity, and this may be due to the vaccination with IBV. Even though the H120 vaccine confers some protection, even though the local chicken operations heavily immunize against IBV, new serotypes and variants continue to arise, making disease control challenging (16).IBV strains differ all over the world in their virulence, tropism, and tissue pathogenicity (31,32). The severity of histopathological lesion and inflammation of the bronchi and bronchopneumonia vary from mild, moderate, and severe lesion, and this contributes to the route of infection, severity of the viral infection, immunological trends of IBV (33) as well as our results showing necrosis and desquamation of the epithelial cell, which are related to the replication of the virus inside cells and tissue (34). Furthermore, the replication of the virus within the lymphoid follicle of the intestine causes vascular changes represented by congestion of the blood vessels, hemorrhage, and hyperemia.

Conclusion:

In the current investigation, it was found that the antibody level against field IBV can be successfully employed for early and quick screening of IBV detection and that the ELISA assay is a diagnostic process that is less complicated than other tests, less expensive, and requires only the barest minimum of lab resources. IBv. infection has continued to be a problem in the poultry farms because of the insufficiency of the vaccine specificity against endemic strains of IBV in Iraq, so current mass application strategies for IBV vaccines are incompetent and result in vaccination collapses. Finally, in our investigated area, additional research is required on virus isolation and molecular characterization of the target gene or genes.

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Conflict of Interest:

No conflict of interest is disclosed by the authors.

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References

1 .Cavanagh D. Coronaviruses in poultry and other birds. Avian Pathol. 2005 Dec;34(6):439-48.https://doi.org/10.1080/03079450500367682

2 .Cavanagh D. The application of nucleic acid-based technology to avian virus detection and characterization. Avian Pathol. 2001 Dec;30(6):581-98.https://doi.org/10.1080/03079450120092071

3 .Al-Shekaili T, Baylis M, Ganapathy K. Molecular detection of infectious bronchitis and avian metapneumoviruses in Oman backyard poultry. Res Vet Sci. 2015 Apr;99:46-52.https://doi.org/10.1016/j.rvsc.2014.12.018

4 .Liu IL, Lin YC, Jian CZ, Cheng IC, Chen HW. A novel immunochromatographic strip for antigen detection of avian infectious bronchitis virus. Int J Mol Sci. 2019 May;20(9):2216.https://doi.org/10.3390/ijms20092216

5 .Cavanagh D, Naqi SA. Diseases of Poultry. Ames: Iowa State University Press; 2003. p. 101-119.

6 .Cavanagh D, Gelb JJ. Infectious bronchitis. In: Saif YM, editor. Diseases of poultry. Ames: Blackwell Publishing; 2008. p. 117-135 .

7 .Yan SH, Chen Y, Zhao J, Xu G, Zhao Y, Zhang GZ. Pathogenicity of a TW-like strain of infectious bronchitis virus and evaluation of the protection induced against it by a QX-like strain. Front Microbiol. 2016 Oct;7:1653.https://doi.org/10.3389/fmicb.2016.01653

8 .Oulianne M, Brash ML, Charlton BR, Fitz-Coy SH, Julian RJ, Jackwood MW, et al. Avian Disease Manual. 7th ed. Jacksonville, Florida: American Association of Avian Pathologists, Inc.; 2012 .

9 .Jackwood MW. Review of infectious bronchitis virus around the world. Avian Dis. 2012 Dec;56(4):634-41.https://doi.org/10.1637/10227-043012-Review.1

10 .Mahmood MS, Siddique M, Hussain I. Carrier state studies of infectious bronchitis virus in asymptomatic layers in Pakistan. Int J Poultry Sci. 2004;3:547-9.https://doi.org/10.3923/ijps.2004.547.549

11 .Lim TH, Kim MS, Jang JH, Lee DH, Park JK, Youn HN, et al. Live attenuated nephropathogenic infectious bronchitis virus vaccine provides broad cross protection against new variant strains. Poultry Sci. 2012 Jan;91(1):89-94.https://doi.org/10.3382/ps.2011-01739

12 .Case JT, Ardans AA, Bolton DC, Reynolds BJ. Optimization of parameters for detecting antibodies against infectious bronchitis virus using an enzyme-linked immunosorbent assay: temporal response to vaccination and challenge with live virus. Avian Dis. 1983 Jan;27:196-210.https://doi.org/10.2307/1590385

13 .Wang X, Schnitzlein WM, Tripathy DN, Girshick T, Khan MI. Construction and immunogenicity studies of recombinant fowl poxvirus containing the S1 gene of Massachusetts 41 strain of infectious bronchitis virus. Avian Dis. 2002 Oct;46(4):831-8.https://doi.org/10.1637/0005-2086(2002)046[0831:CAISOR]2.0.CO;2

14 .Balestrin E, Fraga AP, Ikuta N, Canal CW, Fonseca AS, Lunge VR. Infectious bronchitis virus in different avian physiological systems-a field study in Brazilian poultry flocks. Poultry Sci. 2014 Aug;93(8):1922-9.https://doi.org/10.3382/ps.2014-03875

15 .Al-Dabhawe AH, Kadhim HM, Samaka HM. Molecular detection of infectious bronchitis virus and its relation with avian influenza virus (H9) and Mycoplasma gallisepticum from different geographical regions in Iraq. Iraqi J Vet Sci. 2013 Jul;27(2).https://doi.org/10.33899/ijvs.2013.82811

16 .Jordan B. Vaccination against infectious bronchitis virus: a continuous challenge. Vet Microbiol. 2017 Jul;206:137-43.https://doi.org/10.1016/j.vetmic.2017.01.002

17 .De Wit JJ, Cook JK. Factors influencing the outcome of infectious bronchitis vaccination and challenge experiments. Avian Pathol. 2014 Nov;43(6):485-97.https://doi.org/10.1080/03079457.2014.974504

18 .Seger W, Ghalyanchi Langeroudi A, Karimi V, Madadgar O, Marandi MV, Hashemzadeh M. Genotyping of infectious bronchitis viruses from broiler farms in Iraq during 2014-2015. Arch Virol. 2016 May;161(5):1229-37.https://doi.org/10.1007/s00705-016-2790-2

19 .Al-bawi F, Abady NR. Comparative study of phylogenetic analysis of S1 gene of infectious bronchitis virus isolates from Iraq with the selected strains. Plant Arch. 2019;19(2):1904-8.https://doi.org/10.35248/1948-5964.19.11.183

20 .Casais R, Dove B, Cavanagh D, Britton P. Recombinant avian infectious bronchitis virus expressing a heterologous spike gene demonstrates that the spike protein is a determinant of cell tropism. J Virol. 2003 Aug;77(16):9084-9.https://doi.org/10.1128/JVI.77.16.9084-9089.2003

21 .De Wit JJ, Cook JK, Van der Heijden HM. Infectious bronchitis virus variants: a review of the history, current situation and control measures. Avian Pathol.

Al-Qadisiyah Journal of Veterinary Medicine Sciences
(P-ISSN 1818-5746/ E-ISSN 2313-4429)
www.qu.edu.iq/journalvm

2011 Jun;40(3):223-35.https://doi.org/10.1080/03079457.2011.566260

22 .De Wit JJ, Mekkes DR, Koch G, Westenbrink F. Detection of specific IgM antibodies to infectious bronchitis virus by an antibody-capture ELISA. Avian Pathol. 1998 Apr;27(2):155-60.https://doi.org/10.1080/03079459808419317

23 .Bronzoni RV, Fatima M, Montassier S, Pereira GT, Gama NM, Sakai V, et al. Detection of infectious bronchitis virus and specific anti-viral antibodies using a Concanavalin A-Sandwich-ELISA. Viral Immunol. 2005 Sep;18(3):569-78.https://doi.org/10.1089/vim.2005.18.569

24 .Soliman IMA, Salama E, Abdel Rahman NS, Ebied ME, Ahmed HA, El-Mahdy SS. Quantification of antibodies in sera of vaccinated chickens against infectious bronchitis virus by haemagglutination inhibition test. Zagazig Vet J. 2014 Sep;42(3):217-24.https://doi.org/10.21608/zvjz.2014.60195

25 .Monreal G, Bauer HJ, Wiegmann J. Comparison of the enzyme-linked immunosorbent assay (ELISA), haemagglutination inhibition test, and agar gel precipitation test for detection of antibodies to avian infectious bronchitis virus. Avian Pathol. 1985 Jul;14(3):421-34.https://doi.org/10.1080/03079458508436243

26 .Ignjatovic J, Sapats S. Avian infectious bronchitis virus. Rev Sci Tech. 2000;19(2).https://doi.org/10.20506/rst.19.2.1228

27 .De Wit JJ. Detection of infectious bronchitis virus. Avian Pathol. 2000 Apr;29(2):71-93.https://doi.org/10.1080/03079450094108

28 .Bande F, Arshad SS, Hair Bejo M, Moeini H, Omar AR. Progress and challenges toward the development of vaccines against avian infectious bronchitis. J Immunol Res. 2015 Oct;2015.https://doi.org/10.1155/2015/424860

29 .Ramos JU, Panegossi LC, Fonseca LE, de Carvalho RG, Cardoso TC. Infectious bronchitis virus circulation among poultry flocks inside Atlantic biome forest, Northwestern São Paulo, Brazil. Pubvet. 2020;14(12).https://doi.org/10.31533/pubvet.v14n12a715.1-5

30 .Cook J. Poultry diseases. New York: WB Saunders; 2002. p. 298-306.

31 .Abro SH, Renström LH, Ullman K, Isaksson M, Zohari S, Jansson DS, et al. Emergence of novel strains of avian infectious bronchitis virus in Sweden. Vet Microbiol. 2012 Mar;155(2-4):237-46.https://doi.org/10.1016/j.vetmic.2011.09.022

32 .Cheng J, Huo C, Zhao J, Liu T, Li X, Yan S, et al. Pathogenicity differences between QX-like and Mass-type infectious bronchitis viruses. Vet Microbiol. 2018 Jan;213:129-35.https://doi.org/10.1016/j.vetmic.2017.11.027

33 .Yan SH, Chen Y, Zhao J, Xu G, Zhao Y, Zhang GZ. Pathogenicity of a TW-like strain of infectious bronchitis virus and evaluation of the protection induced against it by a QX-like strain. Front Microbiol. 2016 Oct;7:1653.https://doi.org/10.3389/fmicb.2016.01653

34. Lee CW, Brown C, Hilt DA, Jackwood MW. Nephropathogenesis of chickens experimentally infected with various strains of infectious bronchitis virus. J Vet Med Sci. 2004 Jul;66(7):835-40.https://doi.org/10.1292/jvms.66.835