FEED APPLICATION OF DATE PALM KERNEL EXTRACT TO ENHANCE IMMUNE RESPONSE AND MODIFY HISTOLOGICAL CHANGES IN BROILER CHICKENS CHALLENGED WITH INFECTIOUS BRONCHITIS VIRUS

A.A.A. Embaby[1], Samar A. El-Masry[1], A. Galal[2] and Khaled A. El-Dougdoug[1]

[1]Department of Microbiology, Faculty of Agriculture, Ain shams University Egypt

[2]Department of Poultry Production, Faculty of Agriculture, Ain shams University, Egypt.

Corresponding author's E-mail: ahmed.atia@agr.asu.edu.eg , Cellular phone +201002628589

(*Received 8/5/2024, accepted 12/8/2024*)

SUMMARY

Avian infectious bronchitis (IB) disease is caused by avian infectious bronchitis virus (IBV) which causes significant economic losses to the poultry industry worldwide. The aim of this study is to evaluate the dietary ameliorative effect of date palm kernel (DPK) powder to increase the immunity and modify histological changes in broiler chickens challenged with the infectious bronchitis virus. 500 broiler chicks were allotted to challenged and unchallenged groups in a completely randomized design. The unchallenged groups were randomly divided into 2 feed treatments (Control- non-supplemented and control+ supplemented with 1% DPK from 1-35 days of age (DOA), while challenged groups were divided into 4 treatments as follows: Challenged non-supplemented; Challenged + 1% DPK from 1-21 DOA; Challenged+ 1% DPK from 21-35 DOA; and challenged + 1% DPK from 1-35 DOA. At 21 days of age, chicks of challenged groups were nasal inoculated with IBV isolated from infected birds. At 24 hours post inoculation, the morbidity rate was determined according to clinical signs of infection. At 35 days, blood samples were collected from 6 chickens from the jugular vein, and then slaughtered, and macroscopical and histological examinations were done. Serum concentrations of IL-6, IgG, and IgM were determined. The health status of the trachea showed hemorrhage and edema in challenged chickens. Also, renal inflammation, hemorrhage, and uric acid accumulation were observed. Furthermore, challenged chickens showed necrosis and degeneration of renal and lung tissues. The highest morbidity rate was observed in the challenge non-supplemented group (T3), followed by chickens in treatments T4, T5, and T6 compared with unchallenged control chickens. The health status of not challenged chicks supplemented or non-supplemented with kernel date palm extract had significantly better IL-6 concentrations, followed by challenged-supplemented chicks, compared with challenged-non-supplemented chicks that had the lowest concentrations. Similar trends were recorded for the serum concentration of total immunoglobulins and their fractions. In general, dietary supplementation with DPK had a positive effect on inflammatory reactions and stimulated the innate immune responses of chickens during the IBV challenge. Conclusion: It was concluded that dietary addition of DPK at 1% led to highly significant increase in the chicken immune response, a remarkable modification in histological changes in renal and lung tissues, and a decrease in the morbidity rate.

***Keywords**: date palm kernel, immunoglobulin, interlukin-6, infectious bronchitis virus, broiler chickens.*

INTRODUCTION

Avian infectious bronchitis (IB): is one of the most important agents in chickens that causes respiratory disease in the world and causes extensive economic losses in the poultry industry (Boroomand *et al*., 2012). IB is a common, acute and highly infectious disease in chickens that caused by coronavirus IBV (Bentley *et al*., 2013). Infectious bronchitis virus (IBV) is a gamma coronavirus in the subfamily Coronavirinae and family Coronaviridae. (El-Fetouh *et al*., 2016). Morbidity due to IBV infection can reach up to 100%. Mortality rate may range from 25 to 30% in young chicks but may increase to 80% as a result of factors that are host-associated (age, immune status), virus-associated

(strain, pathogenicity, virulence, and tissue tropism), or environmental cold and heat stresses, dust, and presence of ammonia (Bande *et al*., 2016).

Antiviral activity: The study according to Chen *et al*., 2020 provides, for the first time, clear evidence that the extract of H. perforatum, containing hyperoside, quercitrin, quercetin, pseudohypericin, and hypericin, possess anti- IBV activities. Furthermore, its anti-IBV effect may be associated with reduced mRNA expression levels of the proinflammatory cytokines IL-6, TNF-α by NF-κB signaling pathway, and related to up-regulate mRNA expression levels of type I interferon through the MDA5 signaling pathway and could be useful for the development of new antiviral agents.

According to the results of the antiviral effect assay, eight extracts were selected for determination of the virucidal effect. The selected extracts of *S. Montana, O. vulgare, M. piperita, M. officinalis, T. vulgaris, H. officinalis, S. officinalis and D. canadense* showed anti-IBV activity. All eight extracts showed an antiviral effect prior to infection. Furthermore, seven of these showed antiviral activity during infection, while only the extract of *S. Montana* showed anti-IBV activity after infection. *P. frutescens, N. cataria, E. purpurea, Ch. nobile and A. foeniculum* showed an antiviral effect only in the first method, while *G. macrorrhizum and A. archangelica* did not show an antiviral effect (Lelešius *et al*., 2019).

Date palm kernel (DPK): Date palm kernel can be added in the diet of livestock for augmenting the nutritional worth (Habib, and Ibrahim, 2009) because it has been rich in protein, fat and dietary fiber; it contains 5.1, 9.0 and 73.1 mg/100 g DPK, respectively (Al-Farsi and Lee, 2008). The current study aims to feed application of date palm kernel extract to enhance immune response and modify histological changes in broiler chickens challenged with Infectious bronchitis virus

MATERIAL AND METHOD

Collection of the diseased birds:

Four samples were collected from commercial poultry flocks from different commercial poultry farms in different regions of Egypt (Dakahlia, Giza, Behera and Sharkia governorates) during October 2021 to October 2022. The clinical signs were difficulty with breathing, huddling under the heat source, coughing, gasping, tracheal rales, nasal discharge, lethargy, watery eyes and mildly swollen sinuses symptoms of IBV disease (Butcher, *et al*., 2017). The birds had a history of previous vaccination with IBV vaccines (IB primer at one day old and MA5 at about 14 days old). The selected farms showed mild to moderate respiratory signs that were recorded during the study. Necropsy was also performed and gross lesions were reported (Cook, 2008).

Detection and isolation of IBV:

Collection and preparation of specimens':

Organ specimens (Trachea, lungs, gut and kidneys) were collected from the diseased birds for virus isolation, identification and histopathological examination. The collected Specimens were fixed in neutral buffered formalin 10 % for. The collected organs were washed in sterile saline, and ten grams tissue of each collected organ was homogenate in sterile saline with percent 10% (w/v) by disrupting the organs tissues using sterile mortar and pestle. The homogenates were centrifuged at 3000 rpm for 10 min. The supernatant was treated with 10000 IU/mL penicillin, 1.0 mg/mL streptomycin. And placed at 4°C for 18-24 hours for the presence of IBV using RT-PCR and virus isolation (Heba *et al*., 2016).

Detection of virus infection:

Specific pathogen free (SPF) embryonated chicken eggs (9-11 days old) were used for inoculation of the prepared samples via the allantoic sac route (0.1 mL, each). Additional two passages were performed from the harvested allantoic fluids. Allantoic fluids and chorioallantoic membranes (CAM) were harvested aseptically 48 hours postinoculation. On an 18 days old embryo, all survived embryos were removed from the incubator and placed at 4°C for 18-24 hours. Embryos were then examined for the presence of IBV infection (Hemida *et al*., 2017). about 14 days old). The selected farms showed mild to moderate respiratory signs that were recorded during the study. Necropsy was also performed, and gross lesions were reported.

Detection of IBV isolates:

Haemagglutination test (HA):

The blood was collected from at least four healthy white chickens aged 6 weeks in equal volumes of AL sever solution and mixed thoroughly and then sterilely aliquoted into 1 ml volumes each into a graduated 1.5 ml micro centrifuge tube. The tubes were centrifuged at 3000 rpm for 15 minutes. The red blood cells (RBCs) were resuspended to the original volume using PBS. The tubes were mixed well and centrifuged again. This process was repeated for an average of 3 times until the supernatant of the centrifuged RBCs became clear and without visible blood plasma. The RBCs were diluted to make a 25% working stock solution and refrigerated. In general, the washed RBCs were usable for 5 to 7 days. During the HA tests, the 25% stock solution was diluted to a 1% working solution.

Haemagglutination test (HA) were done as well as that mentioned by Alexander and Senne, (2008) as the following: 500 µl of phosphate buffer saline solution (PBS) dispensed into each tube of serological test tubes. 500 µl of the allantoic fluid harvested from embryo that died more than 24h after inoculation was placed in the first tube and two-fold dilutions were made across tubes. 500 µl of 1% (v/v) chicken RBCs was dispensed to each tube. The solutions were mixed by tapping the tube gently. The RBCs were allowed to settle for about 40 minutes at room temperature, or for 20 minutes at the 37°C, or for 60 minutes at 4°C.The titration of test should be read to the highest dilution giving complete HA (no streaming); this represents 1 HA unit (HAU) and can be calculated accurately from the initial range of dilutions. The test was done according to method described by Allan and Gough (1974).

Haemagglutination inhibition test (HI) was carried out for identification according to Alexander and Senne, (2008) as the following:

500 µl of phosphate buffer saline solution (PBS) dispensed into each tube of serological test tubes. 500 µl of the polyclonal antibody specific for Newcastle virus kindly provided from veterinary serum and vaccine research and production institute, Abbasia, Cairo, Egypt, was placed in the first tube and two fold dilutions were made across tubes.

4 HAU virus / antigen in 500 µl was added to each tube and the tubes were left for a minimum of 30 minutes at room temperature, or for 20 minutes at the 37°C, or for 60 minutes at 4°C. 500 µl of 1% (v/v) chicken RBCs was added to each tube and, after gentle mixing, the RBCs were allowed to settle for about 40 minutes at room temperature, or for 20 minutes at the 37°C, or for 60 minutes at 4°C.

Isolation of the virus:

Specific pathogen free (SPF) embryonated chicken eggs (9-11 days old) were used for inoculation of the prepared samples via the allantoic sac route (0.1 mL, each). Additional two passages were performed from the harvested allantoic fluids. Allantoic fluids and chorioallantoic membranes (CAM) were harvested aseptically 48 hours postinoculation. On the 18th day of embryo age, all survived embryos were removed from the incubator and placed at 4°C for 18-24 hours. Embryos were then examined for the presence of IBV infection (Hemida *et al*., 2017).

Preparation of samples:

Trachea and Lung organs (5gm) were collected from infected braids showing distinct IBV clinical symptoms were grinded in 25 mL of phosphate buffer using sterilized mortar. The extract of trachea and Lung tissues was centrifuged at 3000 rpm for 10 mints under cooling. The supernatant contenting virus partials were collected in sterilized eppendorf tubes for detection virus by RT-PCR.

Extraction of total RNA:

Method of extraction of RNA according to QIAamp Viral RNA Mini kit handbook (April, 2010).

Diagnostic IBV real-time RT-PCR assay:

This real-time RT-PCR assay used the following primer and probe sequences: IBVRT1 forward primer CTA TCG CCA GGG AAA TGT C, IBVRT2 reverse primer GCG TCC TAG TGC TGT ACC C, IBVRT3 TaqMan probe FAM – CCT GGA AAC GAA CGG TAG ACC CT – TAMRA. The primers sequences are truncated versions of those described by Cavanagh *et al*. (2002) and were previously shown to detect PhCoV (Cavanagh *et al*., 2002). A novel TaqMan® real-time PCR probe that recognizes IBV, turkey coronavirus and pheasant coronavirus sequences was designed and incorporated into the test allowing amplification to be followed in real time. One-step RT-PCR reactions were performed using the Qiagen One Step RT-PCR kit. Each 25 πl reaction contained the following components: 5 πl One-step reaction buffer, 1 πl 25 πM MgCl2, 1 πl 10 mM dNTP mix, 0.8 πM IBVRT1 primer, 0.8 πM IBVRT2

primer, 0.2 πM IBVRT3 TaqMan probe, 0.25 πl RNasin ribonuclease inhibitor (between 5 and 10 units of enzyme), 1 πl one-step enzyme mix, 11.75 πl nuclease-free water and 2 πl extracted nucleic acid. The reactions were run on the following Program using a Stratagene MX3000p real-time PCR instrument: 50oC for 30 min, 95oC for 15 min, followed by 50 cycles at 95oC for 20 s and 50oC for 30 s. Fluorescence data were collected during the 50oC step. This reaction was performed in triplicate on each nucleic acid extract, and samples were deemed to be positive if amplification was recorded in two or more of the triplicate reactions.

Purification of DNA:

Purification of amplicons were done using the QIA quick gel extraction kit (Qiagen, GmbH, and Hilden, Germany). The sequence reactions were performed using genetic analyzer Applied Biosystems 3130 (ABI, USA) by big dye Terminator V3.1 cycle sequencing kit. (Perkin, Elmer, Foster city, CA) (Selim *et al.,* 2013).

DNA sequencing and sequence analysis:

PCR products were purified by Magic PCR Preps DNA Purification System procedure (Promega, USA) and sequenced directly using flanking primers and fmol DNA Cycle Sequencing System (Promega, USA) as described by the manufacturer. The sequences determined reflect the nucleotides that were possessed by the majority of the viral RNAs at each position due to possible heterogeneity of virus population. The isolate was sequenced in both directions using forward and reverse primers in duplicate runs. Sequences having less than 99% reproducibility were not analyzed. Aligned sequences were then cropped to a length of 384 bp S1 gene and then used for phylogenetic studies. Sequence alignments and phylogenetic analysis were performed using MegAlign program version 5.0 with Clustal W method (DNAStar Inc., Madison, WI, USA). The phylogenetic tree generated by MegAlign is a rooted tree, predicted from the multiple sequence alignment. The length of each pair of branches represents the distance between sequence pairs, while the units at the bottom of the tree indicate the number of substitution events (Bochkov *et al*., 2007).

Amplification and sequencing of the S1 gene of IBV:

The genotyping RT-PCR uses a panel of ten forward and eighteen reverse primers. Each primer contains a specific sequence to amplify the S1 gene of certain known IBV strains, together with a generic M13 primer sequence (either an M13 forward or reverse sequence, as appropriate). The primers were designed to amplify the strains of IBV currently in circulation in Europe (Worthington *et al*., 2008), along with other strains such as VICS and V18-91 that are not commonly found in Europe. To produce amplicons for sequencing, one-step RT-PCR reactions were performed using the Qiagen One-Step RT-PCR kit. Each 25 πl reaction contained the following components: 5 πl one-step reaction buffer, 1 πl 25 mM MgCl2, 1 πl 10 mM dNTP mix, 0.4 πM of primer S1for2, 0.4 πM of primer S1rev3, 0.04 πM of each of the remaining primers, 0.25 πl RNasin ribonuclease inhibitor (between 5 and 10 units of enzyme) (Promega, Southampton, Hampshire, UK), 1 πl one-step enzyme mix, 12.75 πl nuclease-free water and 2 πl of extracted nucleic acid. The reactions were run on the following program using an MX3000p real-time PCR instrument: 50OC for 30 min, 95oC for 15 min, followed by 50 cycles at 94oC for 30 s, 54oC for 30 s and 72oC for 30 s. The amplicons generated were sequenced using the M13F (GTAAAACGACGGCCAGTG) and M13R (CAGGAAACAGCTATGACCATG) generic primers. The forward and reverse sequences were aligned, and a 140-bp region of the sequence data generated was compared to a library of 36 sequences of well-characterized IBV strains using the ABI Prism SeqScape v2.6 software (Applied Biosystems). The IBV S1 sequence files were taken from the public access Genbank database and included representatives of the major groups of IBV strains that are currently circulating in Europe (Worthington *et al*., 2008) alongside other IBV strains. A neighbour-joining phylogenetic tree was constructed using the Molecular Evolutionary Genetics Analysis package (MEGA v4) with the Kimura 2-parameter algorithm (Tamura *et al*., 2013).

Translation of nucleotide sequence's amino acids:

Amino acids phylogenetic tree was drawn for the sequenced isolates along with other vaccine and reference strains available in the GenBank database using MEGA version 6 (Tamura *et al.,* 2013). A comparative analysis of deduced amino acids and nucleotide sequences of the HVR3 was created using the CLUSTAL W Multiple Sequence Alignment program, version 1.83 of MegAlign module of Lasergene DNAStar software (Ziegler *et al.,* 2002). The phylogenetic tree analysis was conducted by neighbor –joining method using bootstrap analysis (1000 replications) using Mega 5 software.

Preparation of date palm kernel extract:

Date palm kernel was crushed. Then, an analysis of the components of the date kernel was carried out by HPLC Chromatograph and GC-MS to determine the Compound Structure by Hit Spectrum at The Regional Center for Mycology and Biotechnology Laboratory, Al-Azhar University, Cairo, Egypt.

Gas chromatography–mass spectrometry (GC-MS) system:

The chemical composition of samples were performed using Trace GC1310-ISQ mass spectrometer (Thermo Scientific, Austin, TX, USA) with a direct capillary column TG–5MS. HPLC Chromatograph analysis: The composition of the date palm kernel powder was determined by HPLC - PDA analysis HPLC system. HPLC Column: LC-C18 reversed-phase column (25 cm x 4.6 mm, 5 mm; Sigma), injection volume: 50 µl using auto sampler, Detector: PDA detector (model 1260 infinity). The typical chromatogram recorded at 280 and 340 nm. phenolic acids and flavonoids were tentatively identified and quantified in the date palm kernel powder by comparison with the retention times and UV spectra of standards that were analyzed under identical conditions.

Experimental birds and management:

One hundred and eighty unsexed one-day-old of Cobb 500 broiler chicks were used in this study obtained from a commercial hatchery divided into 6 treatments, each treatment comprised of 30 chicks divided into 3 replicates of ten chicks each as follows: the 1st treatment was without viral nasal treatment and without feed additive (Placebo) (Control). The 2nd treatment was with feed additive treatment only until the end of the trial period. The 3rd treatment was with viral nasal treatment only until the end of the trial period. The 4th treatment was without feed additive treatment until the twenty-one day then adds viral nasal treatment and add feed additive treatment. The 5th treatment was with feed additive treatment until the end of the trial period and adds viral nasal treatment at the day twenty-one. The 6th treatment was with feed additive treatment until the day twenty-one only then adds viral nasal treatment.

Birds care and vaccination: Excreta were removed on a daily basis regimen in insure keeping all birds under the same managerial, hygienic and environmental conditions throughout the entire experimental period (to reduce the smell of ammonia in breeding site). All birds were vaccinated for Colone30 (ND), Bursine Plus (Gumboro), Colone30 (ND) and Lasota.

Measurements and procedures:

At the last day of the study, the data for Broiler performance as Live body weight (LBW), Body weight gain (BWG), feed consumption (FC) and feed conversion ratio (FCR), were calculated and recorded, respectively, for all birds. The slaughter procedure, carcass and dressing percentage were measured. Some sera of samples were subjected biochemical investigations as serum blood Total IgA, IgG, IgM and Total Igs. By an automated clinical chemistry analyzer (Sionthiker SK3002B, Tamil Nadu, India). Serum activities of the selected enzymes are a good indicator for many of functions in broiler chickens (Ghareeb *et al*., 2012). The measure of the level of chicken Interleukin 6, IL-6 ELISA was determined by the method of Nakamura, *et al*., 1991.

Morbidity rate:

After 48 hpi (hour post-inoculation), 10 chickens of each treatment were examined to determine the clinical signs of infection, then morbidity rate was calculated by dividing the total number of disease cases by the total samples (as a percentage).

Gross lesions:

Necropsy was performed, and gross lesions were reported. At 35th day of age, five birds from each treatment were slaughtered and eviscerated, and histopathological changes of the lungs, kidneys, and gut were examined.

Histological examination:

Samples from the right lung, middle lobe of the right kidney and about 2 cm of ileum were taken, fixed in 10% formalin solution for 48 h, then dehydrated with gradual concentrations of ethyl alcohol, followed by gradual concentrations of xylene. Samples were embedded in paraffin wax, sectioned to 4 microns, and stained with hematoxylin and eosin. Slides were examined using an optical microscope supported by a camera (OPTICA) according to Adedapo *et al*. (2009).

Statistical analysis:

The experimental data were analyzed statistically by using the general linear model procedure of the Statistical Analysis System (SAS 2001) (Jones *et al*., 2001). Overall data were analyzed using one-way ANOVA test. Significance of differences between groups was determined using the Duncan Multiple Range Test (Duncan, 1955) test for post-hoc comparisons. Differences were considered significant if $P \leq 0.05$.

RESULTS AND DISCUSSION

Part I: Viral assessments

A-Detection and isolation and of IBV:

1 - Prevalence and detection of virus:

The Infectious bronchitis virus (IBV) caused economic losses in poultry industry (Nguyen *et al*., 2013) The IBV has widely distributed around the world, and many variants have been isolated (Wang *et al*., 1994). A major problem in the poultry industry caused by the appearance of antigenic variants of IBV. Vaccine usage can control outbreaks of IBV but IBV outbreaks continue in vaccinated fowls (Liu *et al*., 2003). In Egypt diagnosis of poultry diseases is based mainly on gross lesions and clinical signs with limited laboratory investigations used for confirmatory diagnosis. Conventional diagnosis of IBV is commonly based on isolation of virus and propagation in embryonating eggs, followed by Haemagglutination test of the harvested allantoic fluids. Since two or three blind passages are often required. IBV was detected in commercial chickens during 2021/2022 based clinical Signs and confirmed by Haemagglutination test in farms of El Dakahlia, El. Giza, El Behera and El Sharkia, Governorates, Egypt. The clinical Signs were related to the rick factors, age, mortality, programmer of vaccination, clinical and postmortem signs were recorded sixty collected diseased chickens in Table (1).

Table (1): Prevalence of Infectious Bronchitis Virus (IBV) based on Signs on poultry, organs, rt-RT-PCR and Haemagglutination of collected diseased birds at winter 2021season.

Governorates	Age (days)	CSP	Clinical Signs on organs **(CSO)**											
			Gut (n=15)			Kidney (n=15)			Trachea (n=15)			Lung (n=15)		
			Signs	HA	PCR	Signs	HA	PCR	Signs	HA	PCR	Signs	HA	PCR
El Dakahlia (n=15)	30	Bd, Rf	SC,BV	+5	+8	H,E, DR	+3	+5	SC, TML	+5	+8	DR	+8	+10
El. Giza (n=15)	28	Gr, C, SND	BV	+2	+5	DR, H	+3	+4	SC	+4	+6	DR	+5	+7
El Behera (n=15)	33	D,BR , GR,C, TR, N, L,WE	SC,BV, DR	+8	+10	H,E, DR	+5	+8	SC, TML,	+6	+10	DR, SC, BV	+12	+16
El Sharqiyah (n=15)	30	C,TR,SNE	SC	+3	+5	DR	+3	+5	SC	+4	+6	DR	+4	+6
Total (n=60)	28-33			+18	+28		+14	+22		+19	+30		+29	+39

Clinical Signs on poultry (CSP) = Breathing difficulty (Bd) , ruffled feathers(Rf) Gasping rales(Gr) , coughing(C) ,sneezing nasal discharge(SND) depression (D), tracheal rales (TR) , nasal(N) , lethargy (L), watery eyes(WE)

On the other hand, the clinical signs were difficulty with breathing, huddling under the heat source, the high rate IBV infection showed in El Behera and El. Giza followed by El Dakahlia and El Sharqiyah Governorates. Four organs samples were collected from IBV infected commercial poultry and showed positive results by Haemagglutination test (HA) so this agreed with Roussan *et al*. 2008 compared with control which negative results. The high HA titer 128 / mL was illustrated in Trachea , Kidneys

followed by low HA titer 4 mL in Gut and Lung organs samples were collected from El Behera and El. Giza followed by El Dakahlia and El Sharqiyah Governorates (table 2) .

Table (2): Prevalence of Infectious Bronchitis Virus (IBV) based on Signs on organs, rt-RT-PCR and Haemagglutination of collected diseased birds at winter 2021season.

Homogenate collected organs		Signs	HA result	End point	HA titer/mL
Control	El Dakahlia	-	-	-	-
	El. Giza	-	-	-	-
	El Behera	-	-	-	-
	El Sharqiyah	-	-	-	-
Trachea (n=4)	El Dakahlia	H, E, DR	+	1/8	8
	El. Giza	SC	+	1/128	128
	El Behera	SC, TML,	++	1/128	128
	El Sharqiyah	SC	+	1/4	4
Kidneys (n=4)	El Dakahlia	H,E, DR	+	1/4	4
	El. Giza	DR,H	+	1/64	64
	El Behera	H,E, DR	++	1/128	128
	El Sharqiyah	DR	+	1/8	8
Lung (n=4)	El Dakahlia	DR	+	1/4	4
	El. Giza	DR	+	1/4	4
	El Behera	DR,SC,BV	++	1/128	128
	El Sharqiyah	DR	+	1/4	4
Gut (n=4)	El Dakahlia	SC,BV	+	1/32	32
	El. Giza	BV	+	1/64	64
	El Behera	SC,BV,DR	++	1/128	128
	El Sharqiyah	SC	+	1/8	8

Clinical Signs on organs (CSO) = slightly congested (SC), Blood vessels (BV), Hemorrhage (H), Enlarged (E), Dark red (DR) and Turbid mucous on lumen (TML).

IBV isolation in embryonated chicken eggs:

The Homogenate collected organs from IB diseased samples (Trachea, Kidneys, Lung and Gut) were cultivated in the embryonated egg (9-11 day) with the rate of ten embryonated chicken eggs as replicates per sample. After incubation period, the inoculated chicken eggs were daily examined until death of embryo. After 72 hours, the allantoic fluid caused the death of 10 from 10 chicken eggs embryos for all organs of the collected samples from El Behera Governorate. On the other hand, Al-Sharqiyah Governorate had the lowest death rate of embryos fertilized chicken eggs for all organs of the samples were collected from the same governorate (Table, 3).

Table (3): Number of death Embryonated chicken eggs (9-11 day) inoculated with Homogenate collected organs collected from different governorates.

Organs	Trachea (n=10)			Kidneys(n=10)			Lung (n=10)			Gut (n=10)		
Incubation period	24 h	48 h	72 h	24 h	48 h	72 h	24 h	48 h	72 h	24 h	48 h	72 h
El Dakahlia	2	4	5	2	2	4	2	3	4	2	5	5
El. Giza	2	2	6	2	2	4	1	4	5	1	5	6
El Behera	4	8	10	4	8	10	4	8	10	7	10	10
El Sharqiyah	0	1	1	0	1	2	1	2	2	0	2	3

Propagation and purification of IBV isolates:

Inoculation and propagation of the virus in embryonated SPF eggs before RNA extraction in order to increase the viral titer in the initial sample material (De Wit, 2000). Infectious bronchitis disease (IBD) can be divided into 3 groups based on their virulence such as lentogenic, mesogenic and velogenic. The isolates differ among themselves in the signs as well as the mortality rate in infected poultry flocks. IBV

isolates were propagated in the embryonated chicken eggs (ECE). 50 ml of viral suspensions were collected from allantoic fluid for each isolate. The propagative viral isolates were purified by the alternative low and high speed centrifugation technique. The pellets obtained after high speed were resuspended in 30 ml of saline solution and its concentrations using UV spectrophotometer 108,107 and 106 for velogenic, mesogenic and lentogenic isolates respectively. The UV spectrums of purified IBV isolates were determined by measuring the absorption of UV using spectrophotometer at wave length from 200 to 300 nm (table.4) and illustrated showed that, Amin at 245 nm was 0.57831, 0.43922 and 0.53825 for Velogenic, Mesogenic and Lentogenic respectively. Amax at 255 nm was 0.021, 0.020 and 0.016 for Velogenic, Mesogenic and Lentogenic respectively, A260/A280 was 1.194, 1.1968, and 1.1938 for Velogenic, Mesogenic and Lentogenic respectively and A280/A260 was 0.837, 0.835, and 0. 8376 for three isolates respectively. The values of A260/A280 ratio indicate to good purification of virus particle for three IBV isolates (table 4).

Table (4): Spectrophotometric data of purified IBV isolates.

IBV Isolates	A_{min} (245nm)	A_{max} (255 nm)	A260 (nm)	A280 (nm)	A260/A280 nm	A280/A260 nm
Velogenic	0.57831	1.021	0.58815	0.49243	1.194	0.837
Mesogenic	0.43922	1.020	0.44525	0.37203	1.1968	0.835
Lentogenic	0.53825	1.016	0.54472	0.45629	1.1938	0.8376

IBV Titration by ELISA assay:

The titration of *IBV* was significantly increased (5861 mg/dL) at 35 days age (Chen *et al*., 2011). On the contrary, date plum was significant decreased IBV titer (140, 150 & 100 mg/dL) in IBV infected Chicken at 35 days' post, and pre 21 dpi - IBV as well as pre, 21 dpi- IBV, then post 21 dpi- IBV respectively (Table 5).

Table (5): Titration of Infectious bronchitis virus (IBV) of infected 21 age broiler chicks supplemented with date palm in diet by ELISA assay.

Virus titer Treatments	ELISA values (OD)		Titer Titer mg/dL	Titer Mean Cut of Titer
Date palm + 21 dpi- Infectious virus + date plum	0.062	-ve	100 a	1340
Date plum + 21 dpi - Infectious virus	0. 092	-ve	140 b	
21 dpi -Infectious virus + date plum	0. 105	-ve	150 b	
Infectious virus	1.181	+ve	5861 c	2388
Negative control	0.065		-ve	
Positive control	0.877		+ve	
Note: OD ≥ 0.154 considered as positive.				
Cut of Titer				853

Immunological blood cells:

The blood picture of chickens exposed to doses of the virus indicates an increase in white blood cells and the percentage of hemoglobin and platelets, while in chickens exposed to doses of the additive only and the substance added with the virus, we find the percentages of blood cells and platelets close to the control (Table 6). These observations were based on a comparison with the control group that was statistically significant. [P < 0.0001] (Samuel *et al.,* 2009).

Table (6): Count of Immunological blood cells in Chicken infected with (IBV) feeding feeder supplemented with KDP

Hematological parameters	T1	T2	T3	T4	T5	T6	Sig
WBCs	109.19[a]	113.43[a]	130.89[c]	126.54[c]	122.64[b]	124.29[b]	0.0001***
HGB gm/dl	11.70[a]	12.26[a]	14.76[a]	13.03[a]	13.33[a]	12.40[a]	0.0060**
PLT	83.33[a]	112.00[a]	170.33[c]	165.00[c]	159.00[c]	136.00[b]	0.0001***

*** (Highly significant), *** (very Highly significant)*

Immunoglobulins:

The Infectious bronchitis virus (IBV) due to significant decrease in count of Immunological blood cells (91 ng/L) compared healthy Chicken (164 ng/L) at 35 days age (Fernando et al., 2015). On the contrary, date palm kernel was significantly increased (372 ng/L) compared healthy Chicken (164 ng/L) at 35 days age. In addition date palm kernel was improved significantly increased Interleukin 6 (IL-6) concentration (218 , 264 & 316 ng/L) in IBV infected Chicken at 35 days post , and pre 21 dpi - IBV as well as pre , 21 dpi- IBV, then post 21 dpi- IBV respectively (Table 7)

Table (7): Concentration of Interleukin 6 in Chicks at 35th of age infected with Infectious bronchitis virus (IBV) of infected feeding feeder supplemented. (IBV) feeding feeder supplemented with DPK.

Inter Leukin- 6 / Treatments	IL-6 at age 35 days (ng/L)	Mode of action
Healthy	164 b	Immunomode rate
Date palm kernel	372 f	
21 dpi -infectious virus	91 a	
21 dpi -Infectious virus + Date palm kernel	218 c	Antivirus
Date palm kernel +21 dpi- Infectious virus	264 d	
Date palm kernel + 21 dpi - Infectious virus + Date palm kernel	316 e	

Table (8): Concentration of Immunoglobulins in Chicken infected with (IBV) supplemented with KDP in diet

Immunoglobulins ng/ml	T1 (Control)	T2 (KDP)	T3 (Virus)	T4 (KDP Pre Virus)	T5 (KDP+Virus)	T6 (Virus pre KDP)	Prob.
IgM	272.00	446.33	76.00	120.00	418.33	385.33	0.0001***
IgG	458.00	834.33	138.33	269.67	682.00	514.00	0.0001***
IgA	474.667	632.333	210.000	475.000	536.333	307.333	0.0001***
Total_Igs	1204.67	1912.99	424.33	864.67	1636.66	1206.67	0.0001***

Rat immunoglobulin M (IgM) ELISA Kit Detection Range = 6.25ng/ml-1600ng/ml.
Rat immunoglobulin A (IgA) ELISA Kit Detection Range = 7.81ng/ml-2000ng/ml.
Rat immunoglobulin G (IgG) ELISA Kit Detection Range = 31.25ng/ml-8000ng/ml.
*** (very highly significant))*

Serological detection of IBV:

Slide Haemagglutination test (Rapid HA Test):

The sample No. three were tested by plate HA test giving positive result while samples No. one and two giving no Haemagglutination activity after 2nd passage in ECE. The supernatant of homogenate collected organs was treated by haemagglutination activity. The results in table (9) showed that,

Table (9): Detection and titration of IBV in homogenate collected organs.

Governorates	Age (days)	CSP	Clinical Signs on organs (CSO)							
			Gut (n=15)		Kidney (n=15)		Trachea (n=15)		Lung (n=15)	
			Signs	HA	Signs	HA	Signs	HA	Signs	HA
El Dakahlia (n=15)	30	Bd, Rf	SC,BV	+5	H,E, DR	+3	SC, TML	+5	DR	+8
El. Giza (n=15)	28	Gr, C, SND	BV	+2	DR,H	+3	SC	+4	DR	+5
El Behera (n=15)	33	D,BR , GR,C,TR, N, L,WE	SC,BV,DR	+8	H,E, DR	+5	SC, TML,	+6	DR, SC, BV	+12
El Sharqiyah (n=15)	30	C,TR,SNE	SC	+3	DR	+3	SC	+4	DR	+4
Total (n=60)	28- 33			+18		+14		+19		+29

(+) = Rosetta shape (-) = Button shape
Clinical Signs on organs (CSO) = slightly congested (SC), Blood vessels (BV), Hemorrhage (H), Enlarged (E), Dark red (DR) and Turbid mucous on lumen (TML).
Homogenate collected organs (n=10) inoculated Embryonated chicken eggs

Conventional RT- PCR for S1 gene of IBV:

The quality of purified RNA – IBV from chicken embryo were confirmed by UV spectrophotometer were 1.5, 1.4 and 1.3 at 260/280 ratio O.D .The RNA concentration was 75, 54 ug for Chs, Chp, respectively. The IBV was detected and identified by S1 gene using RT-PCR in examined field samples. The amplified products showed fragments of 886 belonging to nucleocapsid (S gene) of IBV.

CDNA of RNA – IBV was transcribed to cDNA using RT and complementary primer set (reverse primer, -R 5'-GTGAAAACGCGTTGCAAGTT-3'). The c DNA - RNA IBV was amplified using RT-PCR reaction mixture and specific primer sets and analyzed PCR product using 1.5% agarose gel electrophoresis. The amplified DNA was in the expected size calculated ≈.886 bp. (Fig1).

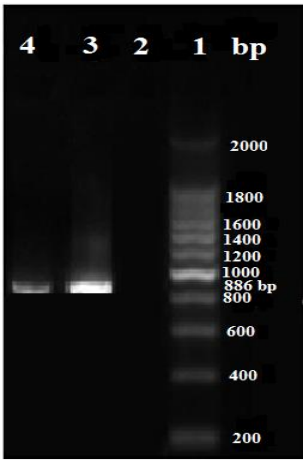


Fig. (1). Electropherogram (1.5%) showing PCR product from IBV-RNA using specific primer sets and compared DNA leader Lane (1). Negative control (2), positive control (3). Velogenic IBV isolate (4); Lentogenic IBV isolate and the arrow to expected size amplified fragment = 886 bp belonging to (S1 gene) of IBV.

The PCR products of S1 gene for IBV were eluted from agarose gel using the gel DNA extraction kit. Amplicons forming the PCR were allowed for sequencing reaction through automated cycle sequencing method. Sequences obtained for each primer for Velogenic IBV isolate had sufficient over layup between them and used to form one continuous sequence (Contag). The partial nucleotide sequences (886 bp) of

S1 gene DNA for Velogenic IBV isolate was done to determine the relationship with other recommended isolates registered in Gen Bank (fig.2).

Partial sequencing of velogenic IBV isolate (886 bp):

CATATGCATGGAGGGATGCTTATGCGGTAGTCTAAGTGTTTCTTTAGAATATAGTAATGA
GAGATTCACAGCAATGTACCGCCGGTGCTATTTATTGGAGTAAGAATTTCAGTGCTGCTT
CTGTGGCTATGACAGCACCTCAAAATGGTATGTCATGGTCAACTTCAGAATTTTGTACGG
CGCACTGCAATTTTACTGATTTTGTAGTATTTGTTACACATTGTTATAAAAGTAGTTCTG
GTTCATGTCCTTTAACAGGTATGATTCCACAGTATTATATTCGTATTTCTGCTATGAGAA
ATAATAGTTTGTTTTATAATTTAACAGTTGCTGTGACTAAATATTCTAGATTTAAGTCGC
TTCAGTGTGTTAATAATATGACATCTGTGTACCTAAACGGCGATCTTGTGTTCACTTCTA
ATGATACTCAAGATGTTAGTGCAGCAGGTGTACATTTTAAGAACGGTGGACCTATAACTT
ATAAGGTTATGAAACAAGTTGATGTCCTAGCTTATTTTGTTAATGGTACAGCACAAGATG
TTATCTTGTGTGATGGTTCACCAAGAGGTCTTTTAGCATGCCAGTATAATACTGGTAATT
TTTCAGATGGTTTCTATCCTTTTACTAATACCAGTTTAGTTAAGGAAAAAGTTTGTTGTT
TATCGTGAAAATAGTGTTAACACCACCTTGGGTTTTGACAAATTTTACATTTTATAATGA
AAAGTAATGCCCCATCCCTAATAATGGTGGTGATTCATACTATTTAGTTTAATACCAAAC
ACATACCAGCCTCAGAGTGGGTATTATGAGTTTTAATTTCTCATTTTCTGAGAAATTTTG
TGTATAAATCTTCCAATATTTCATGTATGGGTC

Fig. (2): Partial nucleotide sequence of S1 gene (886 bp) for isolated IBV.

Sequence analysis based on multiple sequence alignment:

The resulted sequences were compared with an isolated Velogenic IBV using MEGA.4 programmers (Wisconsin, Madison, USA). The sequences of Velogenic IBV isolate aligned for determining the sequence registered in GenBank as shown in Table (10). Multiple sequence alignment (MSA) was displayed in which the corresponding nucleotides occupy the same column. Alignment of multiple genes shows the conserved sites and the percentage of conservation for each position as shown in Table (10).

Table (10): Sequences producing significant alignments

Description	Query Cover	E value	Percent Identities	Accession
Infectious bronchitis virus isolate D1903/21/12_EG S1 spike glycoprotein gene, partial cds	98%	0.0	96.33%	KU238175.1
Infectious bronchitis virus isolate D1456/1/5/10_EG S1 spike glycoprotein gene, partial cds	98%	0.0	96.22%	KU238174.1
Infectious bronchitis virus strain IBV-EG/1212B-SP1-2012 spike glycoprotein gene, partial cds	98%	0.0	96.22%	KU979007.1
Infectious bronchitis virus isolate D2572/2/2/14_EG S1 spike glycoprotein gene, partial cds	98%	0.0	95.76%	KU238178.1
Infectious bronchitis virus isolate D2930/3/1/1/15_EG S1 spike glycoprotein gene, partial cds	98%	0.0	95.30%	KU238179.1

Sequence analysis based on Phylogenetic tree:

Based on MSA analysis, the phylogenetic tree was performed and showed four clusters in which the isolated IBV showed homologous with KU238175.1 (D1903/21/12_EG S1with percentage (96.33%), while KU238174.1 (D1456/1/5/10_EG S1) and KU979007.1 (IBV-EG/1212B-SP1-2012) with percentage (96.22%) and KU238178.1 (D2572/2/2/14_EG S1) with percentage (95.76%), and KU238179.1 (D2930/3/1/1/15_EG S1) with percentage (95.30%), so it was represented as a separate cluster as shown in Figs. (3).

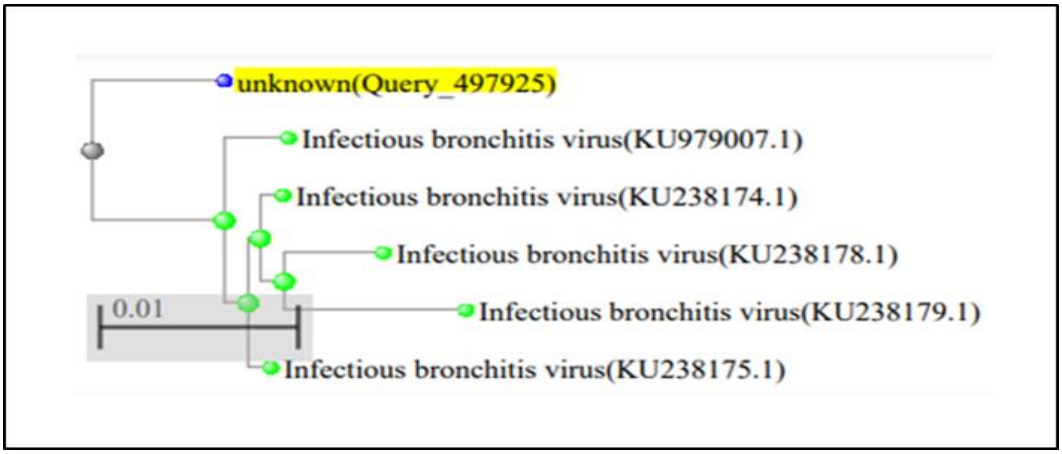


Fig. (3): Phylogenetic tree representing the relationship between isolated Velogenic IBV with Egyptian isolates recorded in gene bank based on Multiple sequence amplified fragment 886 bp alignment of PCR amplified product S1 gene using specific primer of S1 gene with expected size.

```
Query                                                                  14
CATATGCATGGAGGGATGCTTATGCGGTAGTCTAAGTGTTTCTTTAGAATATAGTAATGA  73
KU238175.1  121  .....-...-.....-...-.....-  175
KU238174.1  121  .....-...-.....-...-.....-  175
KU979007.1  121  .....-...-.....A.....-...-.....G.....-  175
KU238178.1  121  .....-...-.....-...-.....-  175
KU238179.1  121  .....-...-.....-...-.....G.....-  175

Query                                                                  74
GAGATTCACAGCAATGTACCGCCGGTGCTATTTATTGGAGTAAGAATTTCAGTGCTGCTT  133
KU238175.1  176  C..G.....  235
KU238174.1  176  C..G.....  235
KU979007.1  176  C..G.....  235
KU238178.1  176  C..G.....  235
KU238179.1  176  C..G.....  235

Query                                                                 134
CTGTGGCTATGACAGCACCTCAAAATGGTATGTCATGGTCAACTTCAGAATTTTGTACGG  193
KU238175.1  236  .....  295
KU238174.1  236  .....  295
KU979007.1  236  .....  295
KU238178.1  236  .....  295
KU238179.1  236  .....C.....  295

Query                                                                 194
CGCACTGCAATTTTACTGATTTTGTAGTATTTGTTACACATTGTTATAAAAGTAGTTCTG  253
KU238175.1  296  .T.....  355
KU238174.1  296  .T.....  355
KU979007.1  296  .....  355
KU238178.1  296  .T.....G.....  355
KU238179.1  296  .T.....  355

Query                                                                 254
GTTCATGTCCTTTAACAGGTATGATTCCACAGTATTATATTCGTATTTCTGCTATGAGAA  313
KU238175.1  356  .....A.....  415
KU238174.1  356  .....  415
KU979007.1  356  .....  415
KU238178.1  356  .....A.....  415
KU238179.1  356  .....A.....  415

Query                                                                 314
ATAATAGTTTGTTTTATAATTTAACAGTTGCTGTGACTAAATATTCTAGATTTAAGTCGC  373
KU238175.1  416  .....  475
```

KU238174.1 416 475
KU979007.1 416 475
KU238178.1 416 475
KU238179.1 416 .....C.....C.....A..... 475

Query 374
TTCAGTGTGTTAATAATATGACATCTGTGTACCTAAACGGCGATCTTGTGTTCACTTCTA 433
KU238175.1 476 535
KU238174.1 476 535
KU979007.1 476 535
KU238178.1 476 .....T.....T.....T..... 535
KU238179.1 476 .....T..... 535

Query **434**
ATGATACTCAAGATGTTAGTGCAGCAGGTGTACATTTTAAGAACGGTGGACCTATAACTT 493
KU238175.1 536 595
KU238174.1 536 595
KU979007.1 536 595
KU238178.1 536 595
KU238179.1 536 .....G..... 595

Query **494**
ATAAGGTTATGAAACAAGTTGATGTCCTAGCTTATTTTGTTAATGGTACAGCACAAGATG 553
KU238175.1 596 655
KU238174.1 596 655
KU979007.1 596 655
KU238178.1 596 655
KU238179.1 596 655

Query **554**
TTATCTTGTGTGATGGTTCACCAAGAGGTCTTTTAGCATGCCAGTATAATACTGGTAATT 613
KU238175.1 656 715
KU238174.1 656 715
KU979007.1 656 715
KU238178.1 656 715
KU238179.1 656 715

Query **614**
TTTCAGATGGTTTCTATCCTTTTACTAATACCAGTTTAGTTAAGGAAAAAGTTTGTTGTT 673
KU238175.1 716 .....G.....-..... 774
KU238174.1 716 .....G.....-..... 774
KU979007.1 716 .....G.....-..... 774
KU238178.1 716 .....G.....-..... 774
KU238179.1 716 .....G.....-..... 774

Query **674**
TATCGTGAAAATAGTGTTAACACCACCTTGGGTTTTGACAAATTTTACATTTTATAATGA 733
KU238175.1 775 .....-..... 833
KU238174.1 775 .....-.....C..... 833
KU979007.1 775 .....-..... 833
KU238178.1 775 .....-.....C..... 833
KU238179.1 775 .....-.....C..... 833

Query **734**
AAAGTAATGCCCCATCCCTAATAATGGTGGTGATTCATACTATTTAGTTTAATACCAAAC 793
KU238175.1 834 ..-.....-..-.....-.....-.....-..... 887
KU238174.1 834 ..-.....-..-.....-.....-.....-..... 887
KU979007.1 834 ..-.....-..-.....-.....-.....-..... 887
KU238178.1 834 ..-.....-..-.....-.....-.....-..... 887
KU238179.1 834 ..-.....-..-.....A..-.....-.....-..... 887

Query **794**
ACATACCAGCCTCAGAGTGGGTATTATGAGTTTTAATTTCTCATTTTCTGAGAAATTTTG 853
KU238175.1 888 ...C..-..-.....T.....A.-.....-.....T.G..... 943
KU238174.1 888 ...C..-..-.....T.....A.-.....-.....T.G..... 943
KU979007.1 888 ...C..-..-.....T.....A.-.....-.....T.G..... 943

```
KU238178.1  888  ......-..-.........T......A.-.................-....T.G.....  943
KU238179.1  888  ...C..-..-.........T......A.-.................-....T.G.....  943

Query         854  TGTATAAATCTTCCAATATTTCATGTATGGGTC  886
KU238175.1  944  .T...........TG..-...-.............  974
KU238174.1  944  .T......C....TG..-...-.............  974
KU979007.1  944  .T......C....TG..-...-.............  974
KU238178.1  944  .T......C....TG..-...-.............  974
KU238179.1  944  .T...........TG..-...-.............  974
```

Figure (4): Alignment between the nucleotide sequences of isolated IBV compared to the similar strains documented in GenBank.

Morbidity rate:

Sneezing and lacrimation were the most common symptoms of the challenged groups with IBV and were observed between 24 and 36 hpi. 70% of the challenged non-supplemented chickens had signs of infection compared with 60% in T4 and T5, while supplemented chickens with SDSP during the experimental period showed 40%. Severe conjunctivitis and nasal discharge (sneezing and lacrimation) with coughing in challenged chickens in T3, T4 and T5 (Fig. 8 a), moderate conjunctivitis and nasal discharge were observed in T6 (Fig.8 b). Normal and healthy chickens were recorded in T1 and T2 (control).

Figure (8). Macroscopic signs of kidneys appeared normal color and size in T1 and T2 (Fig. 8 c). Mild congestion and inflammation in T4 and T5 (Fig. 8b) and moderate effects in T6 while T3 had severe congestion and inflammation with accumulation of uric acid (Fig. 8e). Gross pathological signs of the lungs appeared pink-red in normal color and size in T1 and T2 (Fig. 8l). All challenged chickens appeared to have severe (T3), moderate in T4 and T5 (Fig. 8 n and o), and less severe of congestion and inflammation in T6 (Fig. 8 p). Chickens challenged with IBV had a congestion trachea with necrotic spots (Fig. 8 g) compared with unchallenged chickens (Fig.1f). Duodenum had acute enteritis in challenged Chickens with IBV in T3, T4 and T5 (Fig. 8 i and k) while was less effects in T6 (Fig. 8 j) compared with control (Fig. 8 h).

Histological examination

The transverse section of the cortical region of the kidney showed a preserved Malpighian capsule and normal glomerulus with no pathological abnormality (Fig.5) in control unchallenged chickens. Shrinkage of glomerular tuft and dilation of Bowmen's capsule space in some glomeruli were observed in T3 with degeneration in the epithelial cells that line the convoluted tubules. Similar results were observed in T4 with the infiltration of inflammatory cells and the narrowing of the lumen of convoluted tubules T5 chicken kidneys were more affected by IBV inoculation where renal interstitial congestion and severe necrosis were examined.

Challenged chickens' groups had congestion and degeneration signs compared with unchallenged chickens' groups for the lungs and gut (Fig. 6 and 7). Infectious bronchitis virus (IBV) is a highly contagious disease in chickens. It is a gamma coronavirus. Although IBV primarily affects the respiratory system, it also infects the renal and gastrointestinal systems. Many researchers have demonstrated the pathogenic effects in chickens infected with viruses (Chen *et al*., 2014; El-Fetouh *et al*., 2016; and Hasan *et al*., 2020). The respiratory, urinary, and gut systems are the main target organs of IBV replication. So, similar histopathological lesions were recorded in these organs. Also, IBV is known to replicate in the respiratory tract leading to changes in the muco-cilliary clearance mechanism (Amarasinghe *et al*., 2017). In this study, the pathological effects and macroscopic lesions were observed in challenged chickens and were more severe in challenged non-supplemented chickens. The addition of Siwa date seed powder (SDSP) to chicken diets decreases the morbidity rate and improves chicken health, especially in chickens in group 6 that were challenged with a virus and received a diet supplemented with SDSP during the experiment period (35 days). Less responses to SDSP supplementation were noted in T4 and T5 that were challenged with the virus and received diets supplemented with SDSP before or after the challenge. The presence of flavonoids as antioxidants and antivirals in SDSP may be 31.81 micrograms per am, especially rutin (4.22) and catechin (8.33). The results of the present study agree with El-Fetouh *et al*, (2016) who reported that the infectious bronchitis virus is a major cause and strongly implicated in respiratory and renal problems. Also, Hassan *et al*., (2020) demonstrated that infection with infectious bronchitis virus causes swelling and degeneration of glomeruli of the kidneys.

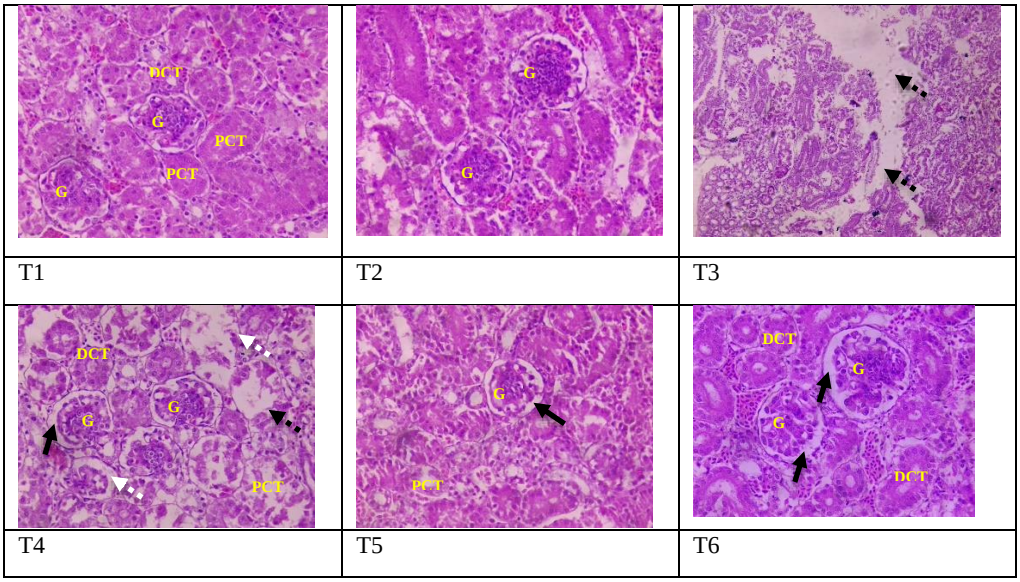


Figure (5): Transverse section in chicken kidney showing normal renal Tubules in non-supplemented unchallenged chickens (T1 and T2). Shrinkage of glomerular and dilation of Bowmen's Capsule space were observed in T4, T5, and T6 (black arrow). Non supplemented challenged chickens in T3 showing necrosis in the renal cortical interstitial tissue (dotted arrows) with degeneration in renal Tubules in T4. G: glomerulus, PCT: proximal convoluted tubules, DCT: distal convoluted tubules. H&E x100.

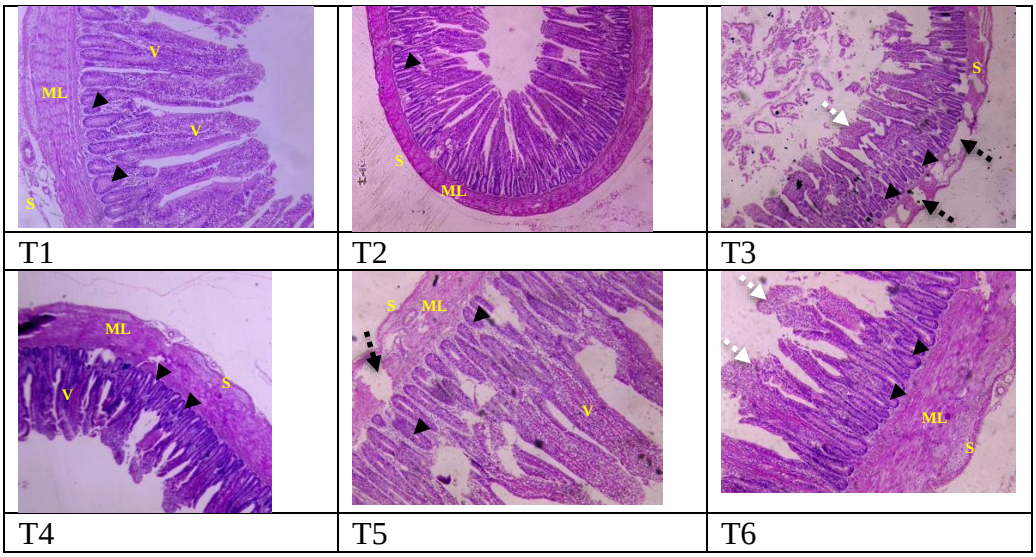


Figure (6): Normal and regular Tissue in duodenum was observed in (T1, T2). Severe degeneration in muscular and Serosa layers and d in T3. Short villi were observed in T4 and T6. Moderate degeneration in muscular and Serosa layers in T5 (dotted arrows). S: serosa, ML; muscular layer, V: villi and head arrow: intestinal gland. H&E x100.

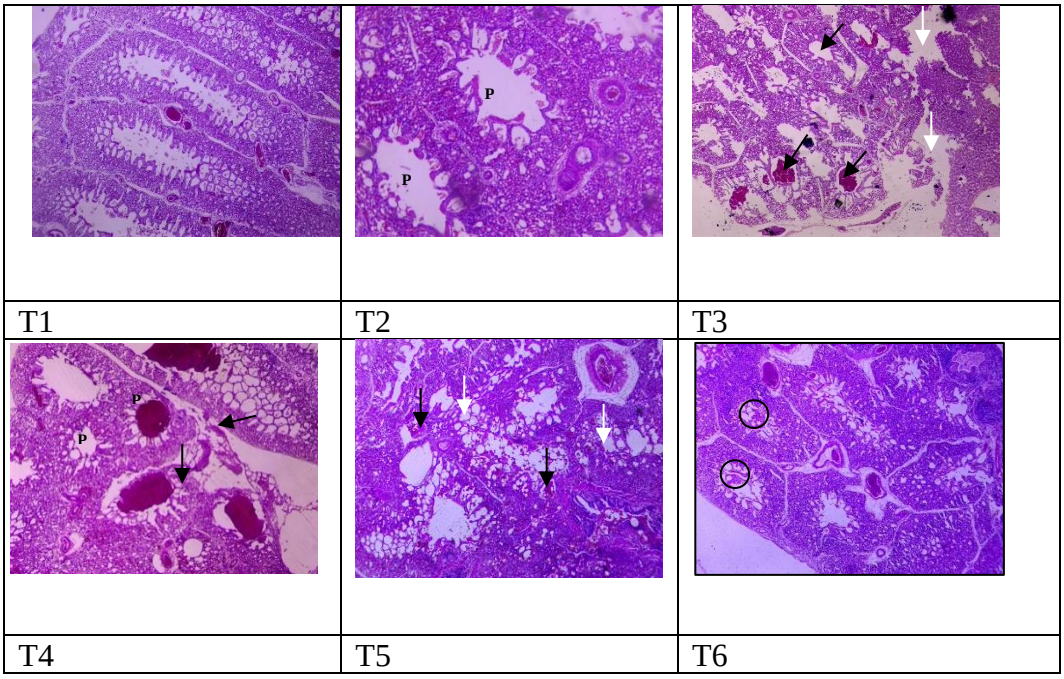
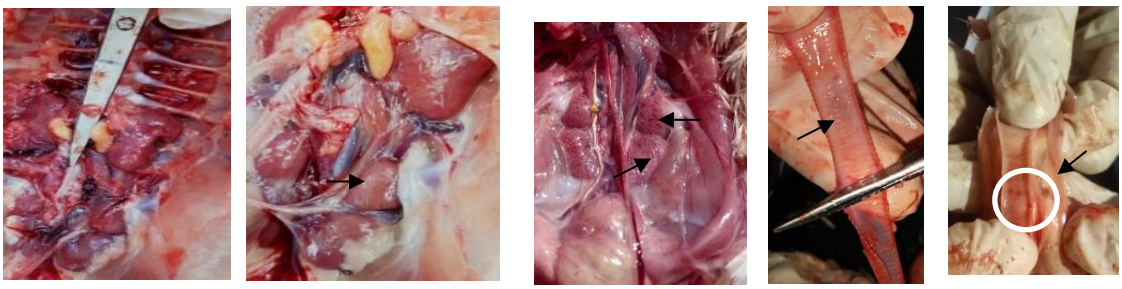


Figure (7): Normal Para bronchi were recorded in T1 and T2. Severe degeneration (black arrow) and hemorrhage (white arrow) in Para bronchi in T3, Moderate hemorrhages T4 with degeneration in T6 (black ring). P: Para bronchi. H&E x100.

Gross pathological observations:

a b

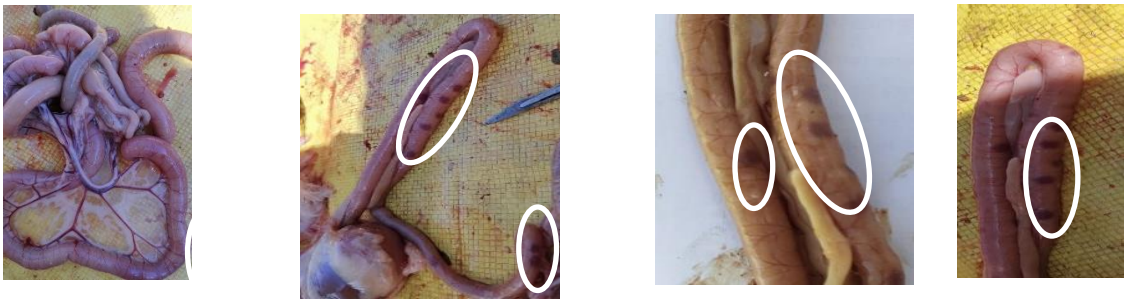
Figure (8): Gross pathological observations. Conjunctivitis (pink-eye) was recorded in supplemented and challenged chickens (T3, T4, and T6) with moderate nasal discharge. (a). Severe sneezing and lacrimation with conjunctivitis and inflammation of eyelid (b)



c d e f g

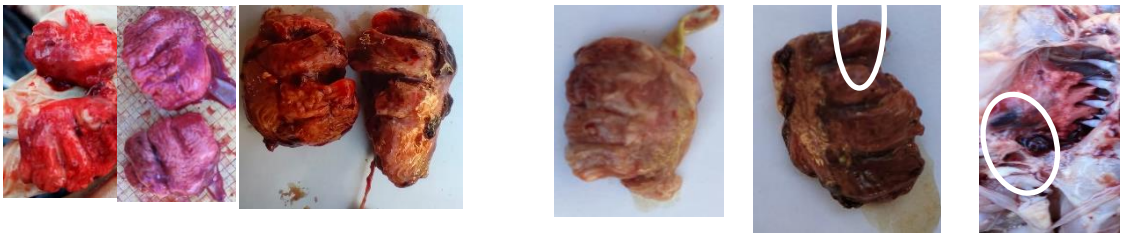
Normal color and size in T1 and T2 (c), inflammation of the kidney lobes in T4, T5, and T6 (d), with accumulation of uric acid granules in T6 (e).11

Gross pathological changes in the trachea of chickens of control chickens show normal structure in T1 and T2 (f). Lungs of challenged chickens show necrotic spots (g).



h i j k

Figure (9): Gross pathological changes in the small intestine of chickens, show normal and healthy duodenum (h) in challenged T1 and T2 compared with severe enteritis in T3 (i). Moderate enteritis of chickens in T4, T5, and T6 (j and k).



l m n o p

Gross pathological changes in the lungs of chickens. The lungs of control chickens show normal appearance in T1 and T2 (l). Lungs of infected chickens show severe congestion, hemorrhage, and consolidation with traces of fibrin in T3 (m), gradual paleness of lungs of chickens in T4, T5, and T6 (n, o, p).

REFERENCES

Adedapo, A. A.; O. M. Mogbojuri and B. O. Emikpe (2009). Safety evaluations of the aqueous extract of the leaves of Moringa oleifera in rats. Journal of medicinal plants Research, 3 (8): 586-591.

Alexander D.J; D.A. Senne (2008). Newcastle disease. Diseases of poultry, 2008 (12): 75-100.

Al-Farsi, M. A., and C.Y. Lee (2008). Optimization of phenolics and dietary fibre extraction from date seeds. *Food chemistry*, *108*(3), 977-985.

Allan, W.H. and Gough, R.E. (1974). A standard haemagglutination inhibition test for Newcastle disease. (1) A comparison of macro and micro methods. Veterinary Record, 95: 120-123.

Amarasinghe, A., Abdul-Cader, M. S., Nazir, S., De Silva Senapathi, U., van der Meer, F., Cork, S. C., 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), e0181801.

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, 2016.

Bentley, K., Armesto, M., and Britton, P. (2013). Infectious bronchitis virus as a vector for the expression of heterologous genes. *PLoS One*, *8*(6), e67875.

Bochkov, Y. A., Tosi, G., Massi, P., and Drygin, V. V. (2007). Phylogenetic analysis of partial S1 and N gene sequences of infectious bronchitis virus isolates from Italy revealed genetic diversity and recombination. *Virus Genes*, *35*, 65-71.

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

Butcher G.D.; D.P. Shapiro and R.D. Miles (2017). Infectious Bronchitis Virus: Classical and Variant Strains. UF/IFAS Extension. 2017 (127): 1-4.

Chen, C.; D.M. Zuckerman; S. Brantley; M. Sharpe; K. Childress; E. Hoiczyk and A.R. Pendleton (2014). Sambucus nigra extracts inhibit infectious bronchitis virus at an early point during replication. BMC veterinary research, 10 (1): 1-12.

Chen, H.W.; C.H. Wang and I.C. Cheng, (2011). A type-specific blocking ELISA for the detection of infectious bronchitis virus antibody. *Journal of virological methods*, *173*(1), 7-12.

Chen, N.; M. Zhou; X. Dong; J. Qu, F.; Gong; Y. Han and L. Zhang (2020). Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. The lancet, 395(10223), 507-513.

Cook, J.K.A. (2008). Coronaviridae. In M. Pattison, P.F. McMullin, J.M. Bradbury and D.J. Alexander (Eds), *Poultry Diseases* (6th edn, pp. 340-349): Saunders Elsevier.

De Wit, J.J. (2000) Detection of infectious bronchitis virus. Avian Pathology, 29, 71 -93.

Duncan D.B. (1955). Multiple Ranges and Multiple F test. In: Biometrics, 11:1-24.

El-Fetouh, A., Mohamed, M. H., 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.

Fernando, F.S.; C.H. Okino; K.R. Silva; C.C. Fernandes; M.C. Gonçalves; M.F. Montassier and H.J. Montassier; (2015). Increased expression of interleukin-6 related to nephritis in chickens challenged with an avian infectious bronchitis virus variant. Pesquisa Veterinária Brasileira, 35 (03): 216-222.

Ghareeb, K.; W.A. Awad and J. Böhm (2012). Ameliorative effect of a microbial feed additive on infectious bronchitis virus antibody titer and stress index in broiler chicks fed deoxynivalenol. Poultry Science, 2012 (91): 800-807.

Habib, H.M. and W.H. Ibrahim (2009). Nutritional quality evaluation of eighteen date pit varieties. Int. J. Food Sci. Nutr., 2009 (60): 99- 111.

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

Heba, N.E.; S.A. Sanaa and S.G. Kasem (2016). Molecular Characterization of Infectious Bronchitis Virus in Chicken. Egypt. J. Vet. Sci., 47 (2): 133-149.

Hemida, M.G.; M.A. Al-Hammadi; A.H.S. Daleb and C.R. Gonsalves (2017). Molecular characterization and phylogenetic analyses of virulent infectious bronchitis viruses isolated from chickens in Eastern Saudi Arabia. *Virusdisease*, *28*, 189-199.

Jones, B.L.; D.S. Nagin and K. Roeder (2001). A SAS procedure based on mixture models for estimating developmental trajectories. Sociological Methods and Research, 29 (3): 374-393.

Lelešius, R.E.; A. Karpovaitė; R. Mickienė; T. Drevinskas; N. Tiso; O. Ragažinskienė; L. Kubilienė; A. Maruška and A. Šalomskas (2019). In vitro antiviral activity of fifteen plant extracts against avian infectious bronchitis virus. BMC Veterinary Research, 15 (178): 1-10.

Liu, H.J.; L.H. Lee; W.L. Shih; M.Y. Lin and M.H. Liao (2003). Detection of infectious bronchitis virus by multiplex polymerase chain reaction and sequence analysis. Journal of Virological Methods, 2003 (109): 31-37.

Nakamura K.; J.K.A. Cook; K. Otsuki; M.B. Huggins and J.A. Frazier (1991). Comparative study of respiratory lesions in two chicken lines of different susceptibility infected with infectious bronchitis virus: histology, ultrastructure and immunohistochemistry. Avian Pathol., 1991 (20): 241

Nguyen, T.T.; H.J. Kwon; I.H. Kim; S.M. Hong; W.J. Seong; J.W. Jang and J.H. Kim (2013). Multiplex nested RT-PCR for detecting avian influenza virus, infectious bronchitis virus and Newcastle disease virus. J. Virol. Method., 2013 (188): 41-46.

Roussan, D.; A.R. Hadda and G. Khawaldeh (2008). "Molecular Survey of Avian Respiratory Pathogens in Commercial Broiler Chicken Flocks with Respiratory Diseases in Jordan". Poultry Science, 2008 (87): 444 – 448.

Samuel, A.O.; O. Olubukola and A.O. Matthew (2009). Hematological and immunological effect on chicken exposed to aflatoxin. Veterinary World, 2 (1): 5-7.

Selim, K.; A.S. Arafa; H.A. Hussein and A.A. El-Sanousi (2013). Molecular characterization of infectious bronchitis viruses isolated from broiler and layer chicken farms in Egypt during 2012. International Journal of Veterinary Science and Medicine, 1 (2): 102-108.

Tamura, K.; G. Stecher; D. Peterson; A. Filipski and S. Kumar (2013). MEGA6: molecular evolutionary genetics analysis version 6.0. Molecular Biology and Evolution, 30 (12): 2725-2729.

Wang, L.; D. Junker; L. Hock; E. Ebiary and E.W. Collisson (1994). Evolutionary implications of genetic variations in the S1 gene of infectious bronchitis virus. Virus Res., 1994 (34): 327-338.

Worthington, K.J.; R.J. Currie and R.C. Jones (2008). A reverse transcriptase-polymerase chain reaction survey of infectious bronchitis virus genotypes in Western Europe from 2002 to 2006. Avian Pathology, 2008 (37): 247-257.

Ziegler, A.; B. Ladman; P. Dunn; A. Schneider; S. Davison; P. Miller; H. Lu; D. Weinstock; M. Salem and R. Eckroade (2002). Nephropathogenic infectious bronchitis in Pennsylvania chickens 1997-2000. Avian Dis, 46 (4): 847-858.

التطبيقات العلفية لمستخلص نواة النخيل لتعزيز الاستجابة المناعية وتعديل التغيرات النسيجية في دجاج انتاج اللحم المصاب بفيروس التهاب الشعب الهوائية المعدي.

أحمد عطية أمبابي [1]، سمر أحمد المصري [1]، أحمد جلال السيد [2]، وخالد الدجدج [1]

[1] قسم الميكروبيولوجى كلية الزراعة جامعة عين شمس, مصر

[2] قسم إنتاج الدواجن، كلية الزراعة، جامعة عين شمس، مصر.

تهدف هذه الدراسة إلى عزل الفيروس المسبب لمرض التهاب الشعب الهوائيه فى الدواجن، وتعريفه ودراسة خصائصه وتطبيق اضافه علفيه (مسحوق نواة البلح) بطرق مختلفه على دجاج سليم ككنترول وعمل اصابات بالفيروس لدجاج اخر لمعرفة مدى مقاومه الدجاج للاصابة بالفيروس بتأثير الماده المضافة.

ولتحقيق هذا الهدف ، تم جمع عينات من الدجاج المصاب بالفيروس من مزارع الدجاج التجارية في ثلاث محافظات مختلفة طوال فترة الفحص (الدقهلية والجيزة والبحيرة) في مصر بين عامي 2021 و2022. وتم جمع إجمالي 240 عينة من الأعضاء (الأمعاء والكلى والقصبة الهوائية والرئتين) لهذه العينات المصابه من 60 قطيعًا مصابًا بالمشاكل التنفسيه، حيث ظهرت عليها علامات تنفسية ونفوق كبير. الكشف عن فيروس التهاب الشعب الهوائية المعدي (IBV) باستخدام تفاعل البلمره المتسلسل للنسخ العكسي واختبار التراص الدموي للدواجن المريضة التي تم جمعها. عزل وتعريف الفيروس ودراسة خصائصه. حيث تم عمل اكثار للفيروسات فى أجنة البيض المخصب وتعريفها سيرولوجيا بأختبار (HA) و اختبار (HI). تم عمل بعض الاختبارات البيولوجيه للفيروسات مثل ICPI و IVPI و MDT.

تم تعريف العزلات بالطيف المرئى باستخدام طيف الأشعه فوق البنفسجه . تم دراسه الشكل المورفولوجى للعزلة المنقاه بتقنيه الميكروسكوب الالكترونى. تم تعريفها جزيئيا باستخدام RT- PCR . تم عمل تتابع نيوكليتيدى للعزله المنقاه وتسجيلها ببنك الجينات.

تم تحضير الماده المضافه وهى مطحون نواة البلح وتم تحليلها لمعرفة مكوناتها باستخدام تقنيات GC-MS و HPLC

تم اجراء التجربه التطبيقيه العلفيه على 180 كتكوت سلالة كب عمر يوم واحد حيث تم تقسيمهم الى 6 مجموعات كل مجموعه 30 كتكوت بثلاثة مكررات (كل مكرر 10 كتاكيت) وتم معاملتهم كالآتى: المجموعة الأولى (T1) التي لم تتغذى على DPK، بل كانت تتغذى على العليقة الأساسية فقط وهى تعتبر ككنترول. المجموعة الثانية (T2) التي تتغذى على DPK، تناولت العليقة الأساسية مع إضافة مسحوق نواة التمر بنسبة 2% (DPK2%). المجموعة الثالثة (T3)تتغذى على العليقة الأساسية فقط ثم تم اصابتها بفيروس التهاب الشعب الهوائية المعدي (IBV) عن طريق التنقيط في العين عند عمر 21 يوما. المجموعة الرابعة (T4) تم تغذية الدواجن على العليقة الأساسية فقط حتى عمر 21 يوماً. ثم تم اصابتها بفيروس التهاب الشعب الهوائية المعدي (IBV) عن طريق التقطير في العين، ثم تم وضع DPK في النظام الغذائي الأساسي حتى نهاية التجربة عند عمر 35 يومًا. في المجموعة الخامسة تم تغذية الدواجن على العليقة الأساسية مضافاً إليها DPK طوال فترة التجربة (35 يوماً). ثم تم اصابتهم بفيروس التهاب الشعب الهوائية المعدي (IBV) عن طريق التقطير في العين عند عمر 21 يومًا. في المجموعة السادسة (T6)، تم تغذية الدواجن على العليقة الأساسية مضافاً إليها DPK حتى عمر 21 يوماً فقط. ثم تم اصابتهم بفيروس التهاب الشعب الهوائية المعدي (IBV) عن طريق تقطيره في العين ثم يتم تغذيتهم على النظام

الغذائي الأساسي المكمل دون إضافة DPK. تم اجراء دراسة بعض القياسات على اداء الدجاج اللاحم لمعرفة تأثير اضافة DPK على العلف لمقاومة الفيروس مثلIgG و IgM و IgA و Total Immunoglobulin .

وتم تقدير تركيز الفيروس بأختبار الآليزا وفى النهايه تم عمل تحليل هيستوباثولوجى لبعض أعضاء العينات مثل (الرئه, الامعاء الدقيقه, الكليه, القصبة الهوائيه) . وقد اوضحت نتائج الدراسه ما يأتى : عينات الدواجن المجمعه من محافظة البحيره كانت اعلى نسبة اصابة من حيث ظهور الاعراض والعلامات المرضيه على الدواجن وكانت اعلى نسبة عدد فى اختبار PCR كانت 13 من 20 وفى اختبار HA كانت 11 من 20 مقارنة بعينات الدقهليه والجيزه. كانت اعلى نسبة نفوق أجنة البيض المخصب فى عزلات البحيره حيث كانت 10 أجنه من اجمالى 10 بيضات مقارنة فى 50 ساعه مقارنة بعزلات الدقهليه 5 أجنه وعزلات الجيزه 4 أجنه فى 72 ساعه. وكانت نتيجة التعريف السيرولوجى لاختبار HA ايجابى فى الثلاث عزلات ولكن كان التركيز فى عزلة البحيره اعلاهم حيث كان 128 مقارنة بعزلات الدقهليه والجيزه كان تركيز كل منهم 64.

وكانت نتيجة التعريف السيرولوجى لاختبار HI ايجابى فى الثلاث عزلات ولكن كان التركيز فى عزلة البحيره اعلاهم حيث كان 64 مقارنة بعزلات الدقهليه والجيزه كان تركيز كل منهم 32. وجاءت نتائج التعريف البيولوجى للعزلات الثلاثه باختبارات ICPI و IVPI و MDT ليثبت ان عزلة البحيره BEI هى عزلة Velogenic شديدة الضراوه بينما عزلات الدقهليه DKI والجيزه GII كل منهم عزلات mesogenic متوسطة الضراوه. تم تضخيم جين الغلاف البروتيني للفيروس IBV بواسطة تقنية تفاعل إنزيم البلمره المتسلسل PCR-RT باستخدام جينات بادئة متخصصة بواسطة الهجرة الكهربائية باستخدام االجاروز جل .٪ 1

لوحظ فى النتائج تأثيرات ايجابيه عند اضافة مسحوق نواة البلح على العليقة الاساسية للدواجن على زيادة وزن الجسم الحى وزيادة معدل استهلاك العلف وايضا معدل التحول الغذائى للدواجن على عكس الدواجن المصابه بالفيروس وجد نقص وانخفاض فى نسب هذه المعدلات السابق ذكرها. كما أدى فيروس التهاب الشعب الهوائية المعدي (IBV) إلى زيادة مستويات كرات الدم البيضاء وPLT في تعداد الدم الكامل، وانخفاض شديد في إجمالي الجلوبيولين المناعي (IgG، IgM وIgA). ومن ناحية التشريح الهيستوباثولوجى وجد انه فى الملاحظات المرضية الجسيمة تم تسجيل التهاب الملتحمة (العين الوردية) في الدجاج المصاب والمعدى (T3، T4، وT6).

ومن ناحية التغيرات المرضية التشريحيه وجد انه في الأمعاء الدقيقة للدجاج تظهر الاثني عشر طبيعيًا وصحيًا في T1 و T2 مقارنة بالشديدة التهاب الأمعاء في T3، تظهر رئتي الدجاج المصاب احتقان شديد ونزيف وتوحيد مع وجود آثار من الفيبرين في T3، وفي كلية الدجاج تظهر الأنابيب الكلوية طبيعية في الدجاج غير المدعم (T1 و T2). وقد لوحظ انكماش الجدر وتوسع مساحة كبسولة بومن في T4، T5، وT6. و يوجد فى دجاج المجموعه T3 يُظهر نخرًا في النسيج الخلالي القشري الكلوي مع انحطاط في الكلى