CLINICO PATHOLOGICAL STUDY AND MOLECULAR DETECTION OF IBD INFECTED BROILERS IN BASRAH PROVINCE

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ABSTRACT

IBD is a highly contagious viral disease which inhibit the immune system of chickens, which is responsible for major economic losses within the poultry industries worldwide. A total of 80 samples were collected from 8 different broiler farms from Qurna, Midyanah, Qarmat Ali, Zubair. Upon the clinical finding and postmortem lesions of the necropsied birds; bursa of fabricius, kidney, Junction between gizzard and proventriculus and thigh muscles were processed for histopathological and molecular detection with PCR. The clinical signs were depression, pasty vent and white dropping. Various overall changes are observed in the bursa, such as swelling, hemorrhages to atrophy in size; In addition, hemorrhages were seen in the thigh muscles. The histopathological changes of Bursa of fabricius showed follicular vacuolation and vascular congestion ;multiples degrees of hemosiderin deposition, and the edema accumulate between follicles and basement membrane ; also, showed moderate infiltration of inflammatory cells and accumulation of inflammatory cells lymphocytes macrophage , plasma cells as well to showed a congestion of the bursal capsule, kidney microscopic findings renal vascular congestion in the cortex and also the medullary area along with the vacuolar degeneration. The suspected tissue samples were assayed using RT-PCR for IBDV targeting VP2 gene. Out of the tested samples 15 were Positives. In spite of using IBD vaccines in different farms of the studied areas; the present study was detected IBD in different areas of Basrah province by PCR and mentioned clinical and histopathological finding, therefore it's necessary to study the sequence analyses of such disease in future.

INTRODUCTION

Infectious bursal disease is a highly contagious viral disease, which affects young chickens. It is immunosuppressive disease of young chickens (1). Which belongs to the Avibirnavirus genus within the Birnaviridae family, this disease involves two types of serotypes: (serotype one and serotype two) (2). It is conjointly known as 'Gumboro disease' per the place wherever its 1st unfold in Gumboro, Delaware, USA. This disease was at the start delineated as avian nephrosis due to the symptoms seen within the kidneys however was later selected infectious bursal disease (IBD), per the structural and histological changes that was seen within the bursa of Fabrice's (3). The IBD causes distraction of lymphoid organs, especially differentiating lymphocytes in the bursa (4).

This disease causes significant direct and indirect economic losses to the poultry industry and poultry farmers around the world (5). Signs of immunosuppression include the inability to respond to vaccines adequately antibody and an increase of the secondary infections (6). Classical forms of disease outbreak may lead to a mortality rate of 50% and in broilers from 3-6 weeks of age that may exceed 3%. Include the main clinical signs depression, watery diarrhea, ruffled feathers, tremors, loss of appetite and death after 2-3 days after the clinical signs onset (7). The preferred method of controlling this disease is through timely vaccination (8).

MATERIALS AND METHODS

Samples collection: The suspected samples were taken from broiler chicks which showed clinical signs related to that of IBD like depression ,pasty vent ,white dropping and lesions as hemorrhage of bursa ,enlargement or edematous bursa and enlarged kidney such samples collected from different areas of Basra province ;Qurna , Midyanah , Qaramt Ali ,Zubair , during the period of extend (August , 2019 – February , 2020). The suspected samples were included bursa of fabricius, kidney, junction between gizzard and proventriculus, thigh muscles then subjected to histopathological preparation and molecular detection with PCR.

IBDV detection by RT-PCR

RNA Extraction: The kit for genomic RNA was used, the favor prep tissue Genomic RNA Extraction Mini Kit (Viral Nucleic Acid Extraction Kit II) animal tissue according to the instructions of manufacturers with modification (Geneaid Company). A set of primers (table 1) were used for the RT-PCR reaction, such primers used to amplification of a 523 bp fragment within vp2 gene. (https://blast.ncbi.nlm.nih.gov/Blast.cgi)

Table (1): The sequence of primers.

Name of primer	Sequence	Tm(c)	Length of primer
VP2-FWD	(5' – GGG GAG AAC TCG TGT TTC AA – 3')	55	523 bp
VP2-REV	(5' – CTT GTT GGC CAT ACG GTC TT – 3')	55	

PCR amplification

Amplification of PCR was used the AccuPower® PCR PreMix kit that contains DNA polymerase, (Bioneer company), dNTPs and reaction buffer in a premixed format that is vacuum-dried into an individual packet. Briefly, the 50 µl of PCR reaction mixture was used in the current study as shown in table (2) .A fragment of 523 bp of the vp2 gene of IBDV amplified by PCR thermo cycling using (Appliedbiosystem) as explained in table (3). Seven µl of PCR products were analyzed by electrophoresis from amplified sample on 2 %agarose gel containing 0.5 ul/25ml ethidium bromide, and run at eighty V for one hour, Images of the gels were photographed on BioDoc Analyze Digital Systems (Biometra, Germany

Table (2): The Reaction Mixture (50 µl) for PCR amplification of *(*IBDV*)*.

No.	Chemical	Amount
1	Master Mix	5.0 µl.
2	Primer Forward (VP2)	1.0 µl.
3	Reverse primer (VP2)	1.0 µl.
4	Template of DNA	10.0 µl.
5	D.W.	33.0 µl.

Table (3): The PCR amplification for VP2 gene

Steps	Temp.	Time	PCR cycles
Pre- Denaturation	95 ºC	5 mins.	1 cycle
Denaturation	95 ºC	20 sec.	35 cycle
Annealing	55 ºC	20 sec.	
Extension	72 ºC	30 sec.	
Final Extension	72 ºC	3 mins	1cycle

Histopathological Study

The Histopathological preparation of the IBDV infected tissues was according to (9).

RESULTS

1-Clinical finding and postmortem: Clinically, some of the visited broiler farms in studied areas in Basrah province were showed clinical signs related to IBD .The most common clinical signs were anorexia, depression. Ruffled feathers, pasty vent and watery white diarrhea. While the lesions were hemorrhage of bursa, enlargement or edematous bursa and enlarged kidney, hemorrhage in thigh muscle and junction between gizzard and proventriculus. Figures (1,2)

Figure (1): Gross section of infected bird showed depression and ruffled feathers.

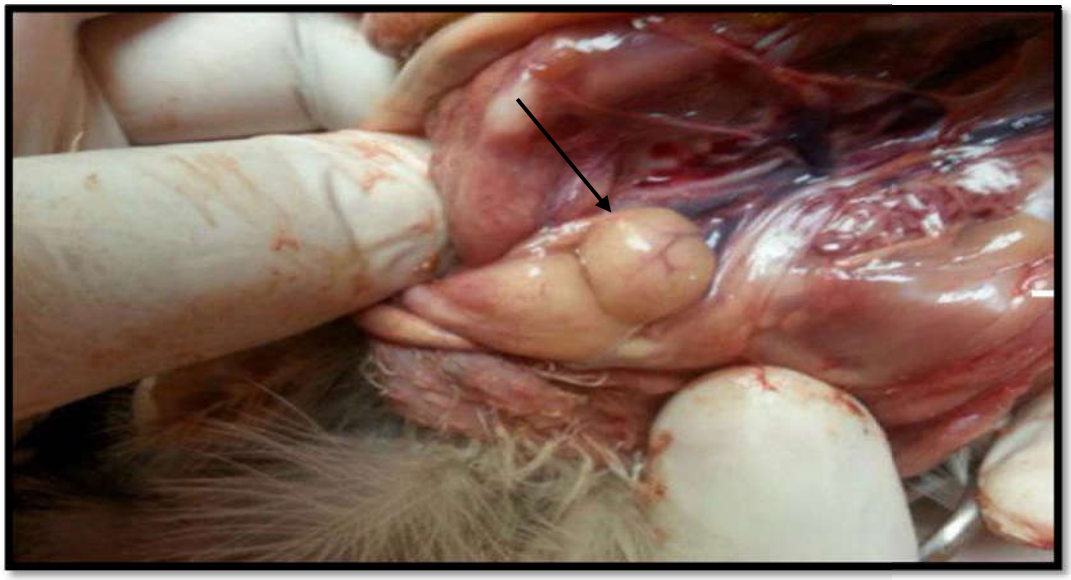

Figure (2): gross section of infected bird showed swelling and enlargement of bursa of fabricius (black arrows).

Molecular Diagnosis

The suspected samples, like bursa of fabricius and kidney were subjected to reverse transcriptase-polymerase chain reaction (RT-PCR) for IBDV detection, by using partial VP2 gene, all (15) RT-PCR positive samples showed specific bands at 523 bp on agarose gel figure (3)

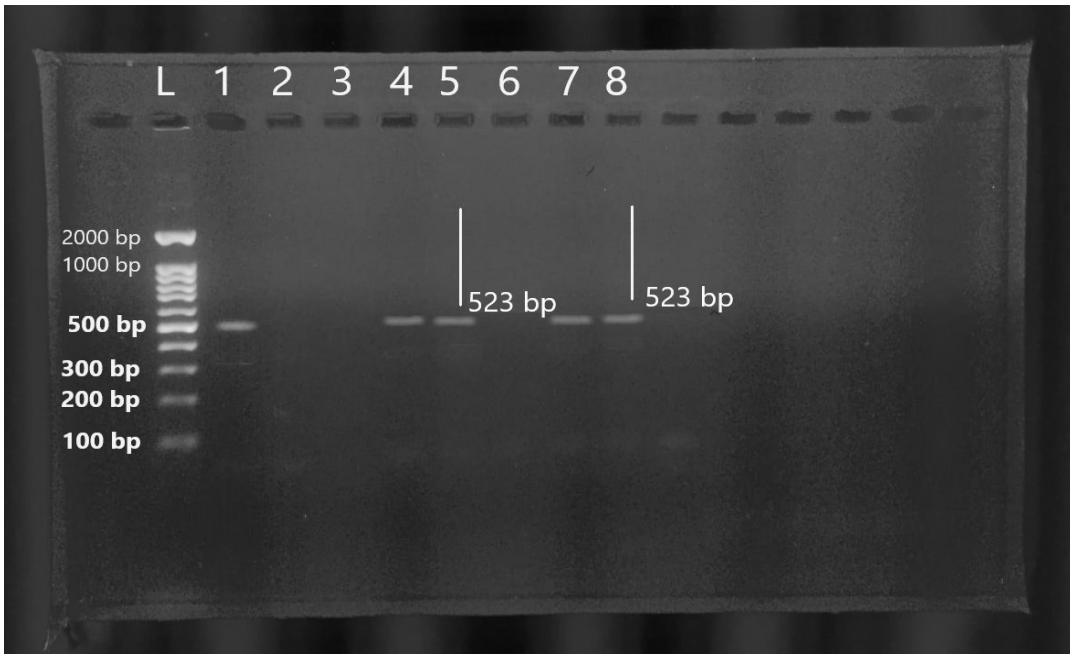

Figure (3): Agarose gel electrophoresis of PCR product L= DNA ladder line (1, 4, 5, 8) positive simple from infected farms, positive control (7).

Histopathological results: The histopathological results of IBD chicken showed different pathological changes in different organs related to chicken infected with IBDV which showed variable changes included in kidney like renal vascular congestion in the cortex and also the medullary area along with the vacuolar degeneration (figure: 4); The histopathological results of Bursa of fabricius showed different types of severity of the pathological changes vary from a follicular vacuolation and vascular congestion (figure: 5), also, showed moderate infiltration of inflammatory cells and accumulation of inflammatory cells lymphocytes macrophage , plasma cells as well to showed a congestion of the bursal capsule (figure:6 **).** The Histopathological results of the junction between proventriculus and gizzard showed a marked area of muscular tissue vascular congestion (figure: 7).

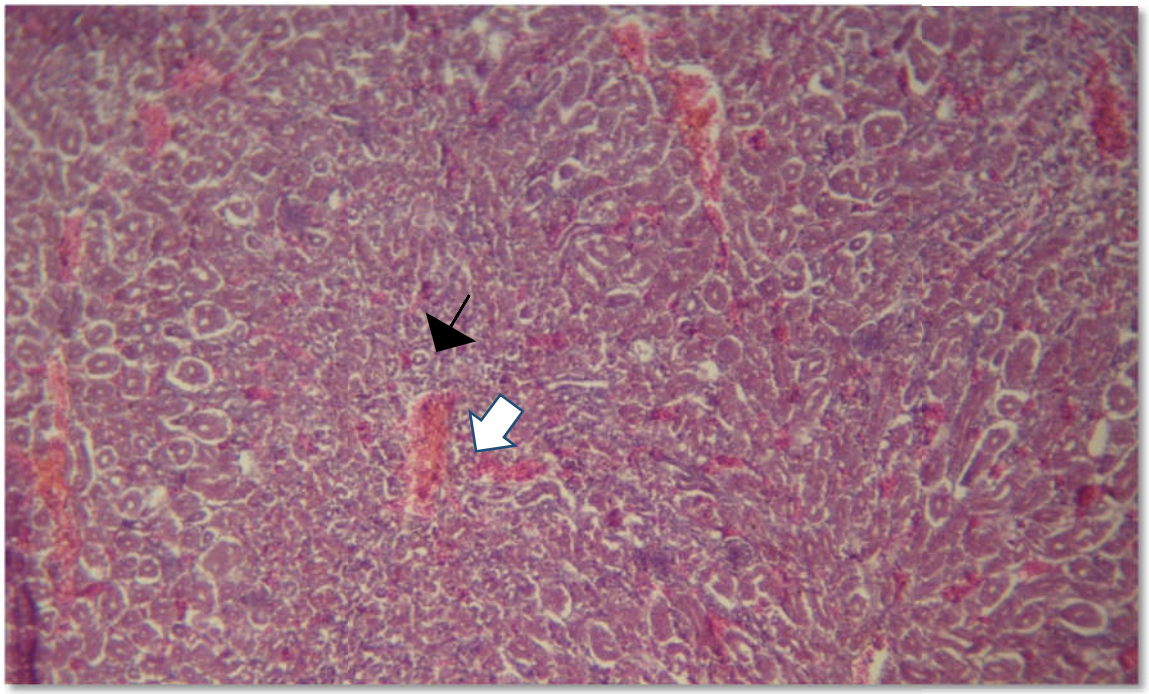

Figure: (4): Histopathologic section of Kidney. Renal vascular congestion (black arrow), interstitial hemorrhage (white arrow) H&Estain 125X

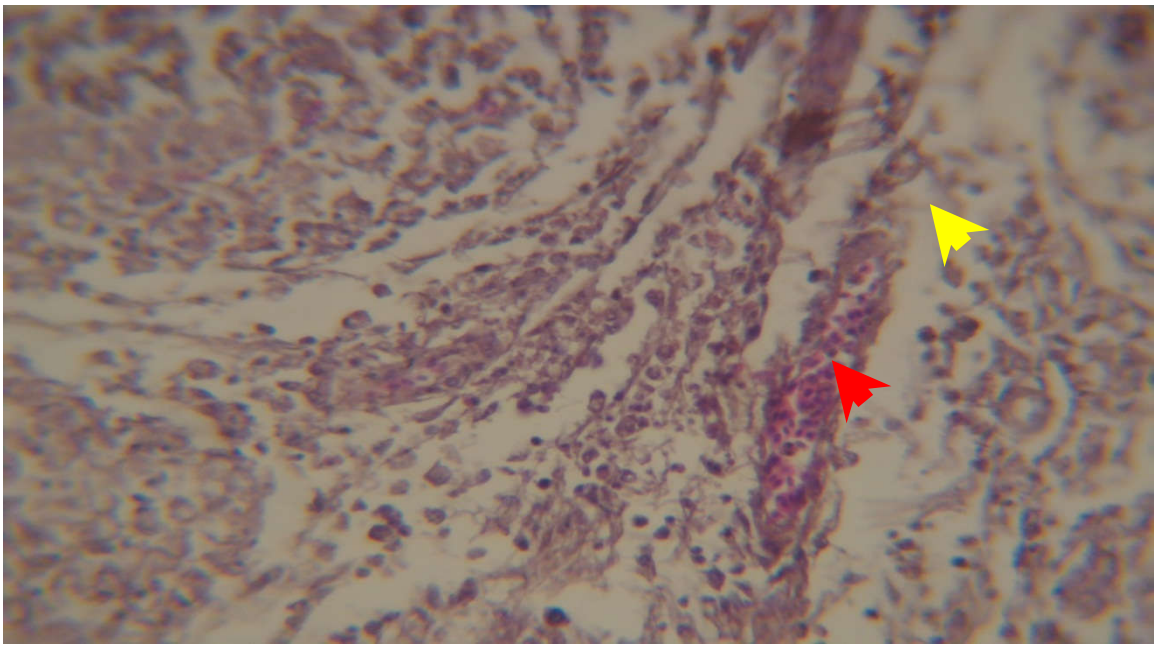

Figure (5). Histopathologic section of Bursa. Bursa reveal vascular congestion (red arrow), Edema in the basement membrane (yellow arrow) (score +1) H&Estain 500X

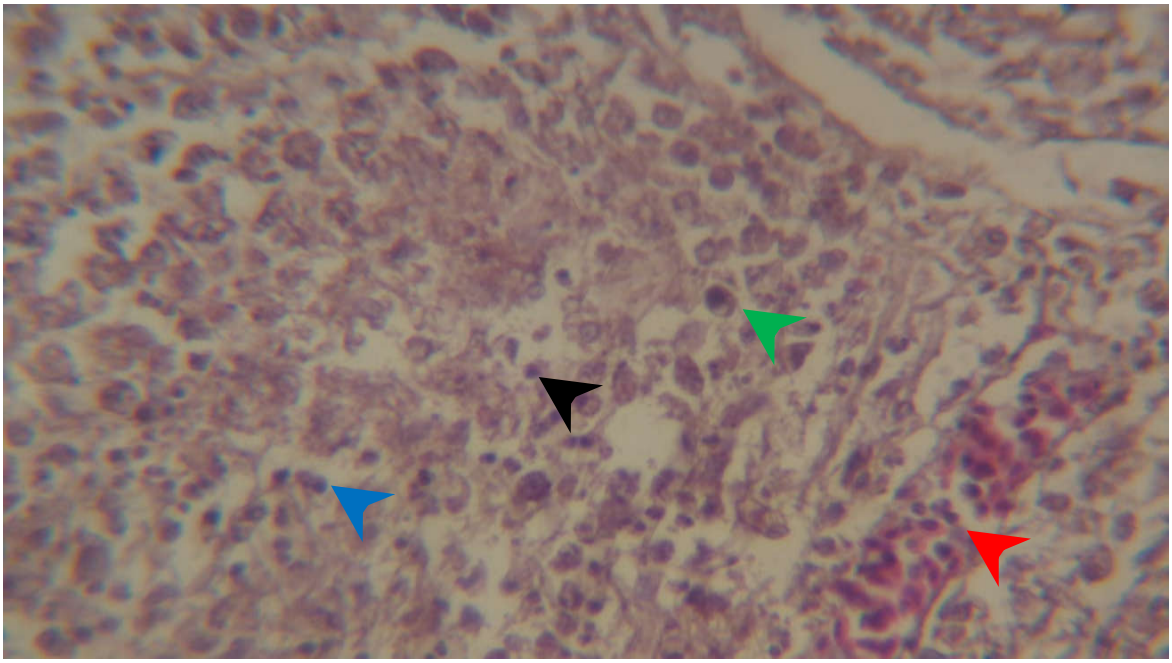

Figure (6). Histopathologic section of Bursa. Bursa reveal vascular congestion (red arrow), accumulation of inflammatory cells, including; lymphocytes (blue arrow), macrophage (green arrow) plasma cells (black arrow). (Score+2) H&E stain 500X

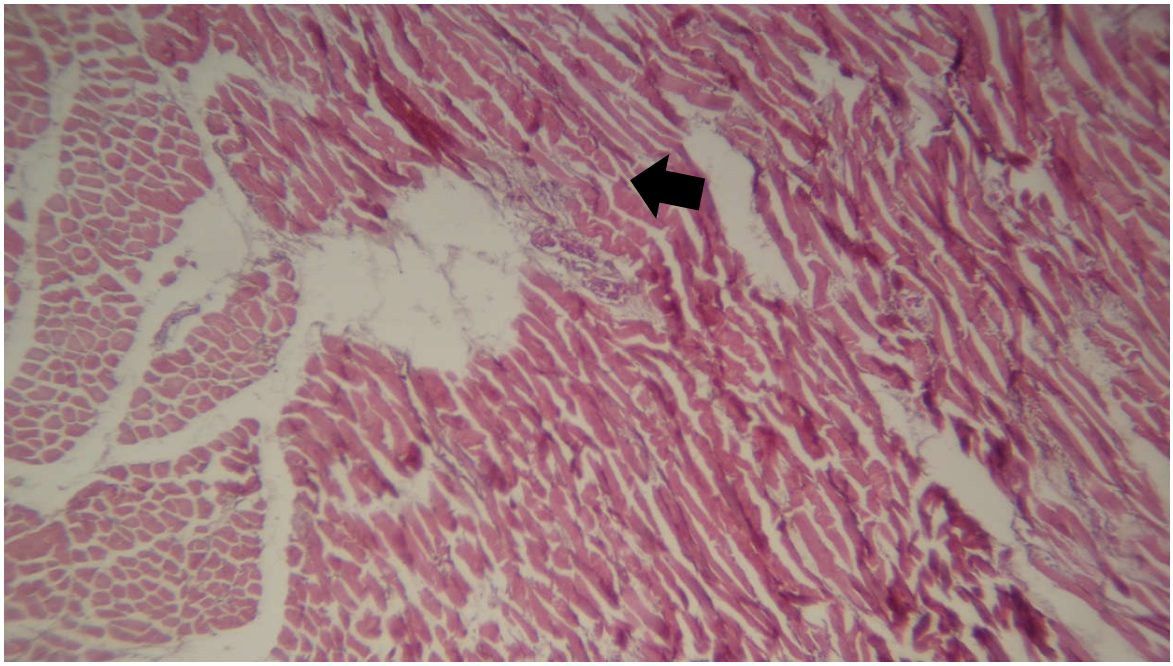

Figure (7): Histopathologic section of Junction between proventriculus and gizzard; muscular tissue shows mild vascular congestion (black arrow) H&E stain 125X

DISCUSSION

IBDv causes the massive economic losses through high mortality and immunological disorder in poultry. The current study showed the infected chicks become loss of Appetite, depressed, ruffled feathers, pasty vent and Watery White diarrhea. The characteristic gross lesions of IBD infected chicks were dehydration of the muscles with ecchymosis hemorrhages and enlargement of kidneys.

The bursa becomes enlarged or edematous and shows pale yellow discoloration. Intra-follicular hemorrhages could also be found and pin point hemorrhages on the thigh muscles, the present results were related to that of (10).who found clinical signs and lesions of IBD infected chicks like depression , pasty vent and changes of bursa of fabricius . these results occur due to the IBV which cause increase in the body temperature leading to decrease movement and depression, also the viral infection causing pasty vent and white dropping may related to nephrosis with enlarged kidneys theses idea agreed with (11) who reported that infectious bursal disease in the suspected birds showed trembling, ruffled feathers, depression, and droopy appearance.

The target organ of IBDV is the bursa of fabricius, the current study showed hemorrhage of bursa, enlargement or edematous bursa, these result occur due to cells infiltration; heterophils and macrophages in the interfollicular space that's may agree with (12). Who reported that hemorrhagic atrophied bursa and enlarged edematous bursa.

The molecular part of our study that performed by using PCR technique which regarded most powerful, tools for diagnosis of IBD, also it is, very sensitive and specific technique to detect such infection in the chicken, were detect presence of IBD in the studied farms ; such idea in line with (2) that reported Several different tests are used to detect the IBD, which do not have ability to detect low levels of IBDV antigens in tissues, while the a fast, specific and sensitive method to detect IBDV is RT-PCR .The current results of of PCR detection were showed positive VP2 gene in the suspected IBD samples, these occur due to the VP2 gene is the major structural protein that builds the viral capsid and used for molecular & epidemiology and phylogenic studies, these result in agree with (13).

The identification of the IBDV genotype was performed per a and therefore the molecular represented RT-PCR aimed toward the hypervariable region of VP2 (14). As a part of the pathological process of IBD that may primarily be explained in histopathological changes in relation to the impact of the virus in many organ among others, liver and excretory organ (15).

Our results were included histopathological changes ; renal vascular congestion in the cortex and also the medullary area along with the vacuolar degeneration &interstitial necrosis; these occur due to aggregation of inflammatory cells in the renal parenchyma as a result of viremia, these result agreed with (16) ,who reported Kidneys vacuolar degeneration of the tubules, glomerular shrinkage and hemorrhages of IBD diseased chicks ,also these results were related to (17) who showed enlargement of kidney in some cases of infectious bursal diseased chicken. The current histopathological study of Bursa of fabricius showed different types of pathological changes vary from a follicular vacuolation, vascular congestion & follicular vacuolation , that may be occur due to site of replication and its target organ of IBDV ; these results were related to the (18) who mentioned Rarefaction of bursal follicles with intermittent infiltration of lympho-mononuclear cells; also the histopathological changes like necrosis and hemorrhages in bursa of fabricius were detected by (15).our results were in line with that of (19), who showed mild

lymphocyte depletion from bursal follicles, congestion of blood vessels،edema & infiltration of mononuclear cells in interfollicular spaces. Also the junction between proventriculus and gizzard showed a marked area of muscular tissue vascular congestion and present of moderate intravascular infiltration of inflammatory cells, these occur duo to viral infection of the chicken causing viremia and then distribution of the virus in the thigh muscle and junction between proventriculus and gizzard leading to replication of the virus these causing dilation of blood vessels as a result of infiltration of inflammatory cells against the viral infection lead to damage of blood vessels, these result agreed with (20), who showed the junction of proventriculus and the gizzard, includes congestion, hemorrhage and infiltration of heterophils at the junction.

دراسة سريرية نسيجية مرضية والتحري الجزيئي لفيروس التهاب جراب فابريشيا المعدي في افراخ فروج اللحم في محافظة البصرة.

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الخلاصة

التهاب جراب فابريشيا المعدي هو مرض شديد العدوى ومثبط للجهاز المناعي في افراخ فروج اللحم ، وهو المسؤول عن الخسائر الاقتصادية الكبيرة في صناعات الدواجن في جميع أنحاء العالم.. تم جمع ٨٠ عينة من ٨ مزارع دجاج تسمين مختلفة من القرنة ، المدينه ، كرمة قرمة علي ، الزبير. اعتمادا على النتائج السريرية والآفات بعد الوفاة للطيور التي تم تشريحها ، تم اخذ العينات من انسجة مختلفة متمثلله ؛جراب فابريشيا ، الكلى ، منطقة الربط بين المعده العضلية والغديه ، والعضلات للكشف عن التغيرات النسيجيه والتحديد الجزيئي بواسطة PCR. العلامات السريرية تضمنت الخمول واسهال مائي ابيض مع تبليل ريش نقطة المجمع. ولوحظت ايضا تغيرات مرضية مختلفة في جراب فابريشيا ، مثل تورم ، نزيف إلى ضمور في الحجم ؛ كما شوهد نزيف على عضلات الفخذ. أظهرت التغيرات المرضية النسيجية لجراب فابريشيا فجوة في الجريبات واحتقان الأوعية الدموية ، وتتضاعف درجات ترسب الهيموسيديرين ، وذمة تتراكم بين الجريبات والغشاء القاعدي. أيضا ، أظهر ارتشاح تسلل معتدل للخلايا الالتهابية وتراكم الخلايا اللمفاوية الضامه، وخلايا البلازما أيضا لإظهار احتقان لجراب فابريسيوس، ونتائج التغيرات المرضية النسيجية للكلية اظهرت احتقان الأوعية الكلوية في القشرة ومنطقة النخاع جنبا إلى جنب مع تنكس أنبوبي فجوي. تم فحص عينات الأنسجة المشتبه بها باستخدام (RT-PCR). تفاعل تسلسلي ل IBDV يستهدف الجين VP2. من بين العينات التي تم اختبارها ، كانت ١٥ إيجابية. على الرغم من استخدام لقاحات IBD على بعض الطيور في المناطق المدروسه؛ تم تسجيل

الدراسة الحالية IBD في مناطق مختلفة من محافظة البصرة بواسطة (PCR) وذكر التقنيات السريرية والمختبرية ، لذلك من الضروري دراسة التحليلات التسلسلية لمثل هذا المرض في المستقبل.

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